CONTROL OF RADIOACTIVE WASTES

THE recent Monaco Conference on Radioactive Waste Disposal (see *Nature* of January 2, p. 1) was held at about the same time as a Bill dealing with the subject was introduced in the House of Lords. At the present time, disposal of radioactive waste in Great Britain, by organizations other than the United Kingdom Atomic Energy Authority, is governed by the same regulations as apply to ordinary domestic and industrial wastes. With the growing use of radioactive substances by a diversity of bodies and because of certain hazards peculiar to the disposal of radioactive wastes, it was realized that these regulations were probably inadequate. In 1956, at the invitation of the Minister of Housing and Local Government, an expert panel was appointed by the Radioactive Substances Committee with the following terms of reference :

"To ascertain the nature and quantity of radioactive waste likely to arise in the foreseeable future ; to advise on the best methods of securing that the waste is disposed of safely ; and to advise whether any new legal provisions or amendments to existing legislation are necessary to ensure safe disposal, and, if so, to advise on the form which the new provisions or amendments should take."

The report of this Committee is contained in a recent White Paper* and the recommendations of the Committee are effectively incorporated in the Radioactive Substances Bill, introduced in the House of Lords on November 13. The Bill provides that the temporary powers by which the discharge of radioactive wastes by the Atomic Energy Authority and other licensed nuclear installations are exercised by the Minister of Housing and Local Government and the Minister of Agriculture, Fisheries and Food, and in Scotland the Secretary of State, will be made permanent. Other users of radioactive materials will be obliged to register their premises with the appropriate Minister (in England and Wales, the Minister of Housing and Local Government and in Scotland, the Minister of State). No disposal of radioactive waste will be permitted except on authorization from the appropriate Minister or Ministers. The Ministry of Housing and Local Government and the Secretary of State for Scotland will, in addition, have power to arrange a national disposal service to handle wastes that cannot safely be dealt with in the area in which they originate. The cost of this service will be chargeable to users of the service.

Both the principles and recommendations emerging from the report of the Committee should be studied by all present and future users of radioactive substances. The views expressed may be briefly summarized. There must be adequate justification for the use of any source of ionizing radiation, no matter how small. Subject to such justification and with suitable precautions, the use of radionuclides should be encouraged. It is not possible to dispose of radioactive wastes without adding to the background irradiation to which the public is exposed, but it is possible to keep this increase well within acceptable limits. Irrespective of cost, it must be ensured that the discharge of radioactive waste does not endanger the health of any individual member of the public, and further, in the terms of the White Paper, "in considering whether a given discharge should or should not be permitted, it is necessary to decide whether the radiation from the discharge, when added to that from all other sources of radiation, is likely to result in a genetic hazard to the nation as a whole ; from this point of view it is as much the concern of, for example, London as of Manchester if the inhabitants of either are exposed to ionizing radiations". It is obvious that local control of discharges would not enable such decisions to be taken, and that a national body staffed by suitable experts would be necessary. When considering the level of permissible discharge, irradiation of the public by other sources, notably normal background, X-ray and fall-out, has to be considered. The Committee suggests that the gonad irradiation arising directly from waste disposal, to which the whole population is exposed, should in no circumstances be permitted to exceed an average dose of 1 r. per person per 30 years, and also emphasizes that it would expect that, with reasonable precautions, the average dose would be maintained much below this level. The recommendation of the Committee for the setting-up of a National Disposal Service in no way suggests that local disposal is unsatisfactory ; on the contrary, local disposal, wherever feasible, is recommended. It is anticipated that the primary function of the National Disposal Service will be to dispose of highly active wastes of long half-life.

A detailed and quantitative picture of the present scale and range of radioactive waste disposal emerges from the report of the Committee. It would appear that, with a few notable exceptions, particularly in the luminizing industry, the hitherto limited controls have so far ensured that radioactive wastes have been disposed of in an acceptable manner. The Committee has been in much greater difficulty, however, when attempting to forecast the quantity of radioactivity to be disposed of in the future. Thus, when considering wastes likely to arise from private reactors, its comment that "we find it difficult to estimate the magnitude of the wastes likely to be produced in these enterprises", emphasizes the need for an organization responsible for radioactive waste disposal on a national basis and for the frequent review of limits and methods of disposal.

* The Control of Radioactive Wastes. Pp. 46. (Cmnd. 884.) (London : H.M. Stationery Office, 1959.) 2*s.* 6*d.* net.

The Committee was in obvious difficulty in suggesting a limit for the irradiation dosage that could be received by the population as a result of waste disposal, in that "there is no agreed permissible genetic dose and there is no agreed allocation of dose to the different ways in which the population is irradiated". Whatever the research effort, it is difficult to imagine a quick answer to this problem. For immediate use, it would appear reasonable to suggest limits, as the Committee has done, which are considerably less than those which informed medical opinion regards as maximal. The report also indicates the need for considerable research in a number of other fields where information is lacking. Thus, when speaking of the discharge of liquid wastes to rivers, the Committee comments that "we know all too little of the absorption on river muds, the absorption into weeds and the take-up by cress". A research inquiry would appear to be required to establish the fate of radionuclides disposed of in sewage systems. It should not be impossible to ascertain what proportion of radionuclides, particularly those such as radiostrontium which are taken up by plants, pass through sewage disposal works and what proportion is retained in the sewage sludge, which may be used as a fertilizer. The report warns of the need for further research before highly active fission products produced as waste from the processing of fuel elements be dumped into the sea. The repeated reference to the lack of experts with the knowledge to deal with the problems of waste disposal poses the question as to whether adequate steps have been taken to ensure the training of sufficient personnel for the efficient working of the proposed central authority.

GEOPHYSICS FOR THE ENGINEER

Geophysical Surveys in Mining, Hydrological and Engineering Projects, 1958

Pp. viii+270. (The Hague, 30 Carel V. Bylandtlaan: European Association of Exploration Geophysicists, 1959.) 16.50 florins; 4.50 dollars.

THOSE who have followed the growth of geophysics since the early 'twenties will remark the happy circumstance that has allowed the publication of this excellent volume of case-histories to occur during Prof. J. M. Bruckshaw's term as president of the European Association of Exploration Geophysicists. Prof. Bruckshaw was a member of the original team which, under Broughton-Edge, produced one of the classics of applied geophysics, based on tests of all the different physical tools against a series of geological structures in Australia.

When it became apparent that geophysical methods could locate potential oil reservoirs, the oil industry application swamped all others. There has been a tendency, as with oil-well drilling, for the tools to be elaborated instrumentally, but, as Prof. Bruckshaw pointed out in his presidential address to the European Association of Exploration Geophysicists, there have been no fundamental changes in the methods that were developed thirty years ago. It is possible that present geophysical methods in the oil industry have already been pushed beyond their economic limits, and it may be opportune to take another look at how the mining and civil engineers use geophysics.

The engineer, whether looking for ore-bodies, locating water-supplies or studying the foundations of buildings or dam sites, cannot afford the luxury of rapid coverage of large areas with routine magnetic, gravity and seismic measurements. He must find the most suitable tool or combination of tools—including the drill—to solve his geological problem. In one respect, of course, the technical problems of the engineer are simplified, since all his underground structures are much shallower than most oil reservoirs, and often cheap and rapid electrical prospecting methods are adequate. However, the case-histories do show a proper appreciation of how geophysical tools should be used in geological work, as, for example, in some of the Rhône valley investigations, where electrical surveys were supplemented by seismic refraction measurements.

It is instructive to the oil geophysicist to read of the wide variety of successful applications of geophysical methods. In Yugoslavia, for example, ancient Roman mining activity is being extended by the exploration of a whole zone of electrical anomalies. In Finland, swamp-covered ore deposits are revealed by magnetic and electromagnetic surveys. The search for uranium-vanadium ores in Colorado in 1940 led to the use of the Geiger counter as a new prospecting tool It is pleasant also for those who have spent a great deal of time listening patiently to the claims of water diviners to see that great success has been achieved in the State of Illinois by using resistivity surveys to locate comparatively small pockets of water-bearing sand and gravel. The drill has shown that in hundreds of surveys the interpretations are 90 per cent correct, a better result than can be obtained by divining.

This collection of past experience should prove of great benefit to civil engineers, since there are many cases, such as the proposed Channel Tunnel or the new Forth Bridge, where the application of geophysical methods can supplement and extrapolate the information obtained traditionally by boring.

T. F. Gaskell

ELEMENTARY PARTICLES

The Theory of Elementary Particles

By Dr. J. Hamilton. (International Series of Monographs on Physics.) Pp. xii+482. (Oxford: Clarendon Press; London: Oxford University Press, 1959.) 75*s*. net.

IT must be said immediately that this is not a narrative book that aims to introduce one to elementary particles or indeed to interest one in them by persuasion at all. It is a book for the professional which deals with the mathematical formalism and techniques that have been found useful for our description of elementary particles and their interactions. The particles themselves, of course, figure and are the subject of a table at its end. They are used, however, rather more to illustrate the formalism than as the subjects of the work itself. The formalism presented is 'useful' in the sense that it is of immediate application in our everyday handling of the elementary particles. There is very little attempt to discuss mathematics in a quasi-philosophical framework as a source of concepts. In other words, this is a book

that tells you how to do the standard calculations about elementary particles using the techniques that have been evolved since the War. It is therefore an extremely useful book and will be of the greatest practical value, particularly as a very thorough and general introduction for theoretical students. It is a book for which a great need has been felt for many years, and it satisfies that need. The writing is of considerable clarity, and it will not be merely the theoretical student who profits by the use of this work. The experimentalist who does not mind a little hard work is well able from this text to put himself in contact with the 'right' way of thinking about particles and their interactions. Helpful examples abound and the author has to be thanked for the large number of 'hints' that he gives in liberal footnotes to sustain the weaker brethren. In short, it is a technical work that will satisfy the professional theoretician without repelling the curious experimentalist. The author is to be both congratulated and thanked.

The early chapters describe the setting up of classical fields and lay stress on general invariance properties of a relativistic origin. Then follow discussions of the quantization of boson and fermion fields and the interaction of fields. This first half of the book is essentially introductory in character and sets out the formalism at some length in a clear and agreeable way. Renormalization methods are then introduced with a discussion of the S-matrix and scattering theory, followed by Heisenberg and interaction representation operators. The Chew-Low theory is discussed in some detail, and there is a good treatment of dispersion relations. Throughout the book the author rightly stresses the relation between the invariance properties and selection rules, and these are discussed at some length. There are final chapters on polarization analysis and the foundations of field quantization.

This is basically a book to be learnt from and to be used rather than one purporting to summarize the state of play. It will be widely appreciated.

D. H. WILKINSON

PETROLEUM INDUSTRY

Advances in Petroleum Chemistry and Refining
Vol. 1. Pp. xv+641. Vol. 2. Pp. xviii+634. Edited by Kenneth A. Kobe and John J. McKetta, Jr. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1959.) 109*s*. per volume.

THE petroleum industry has always maintained large and active research and development organizations, the contribution of which to the advancement of science is published in patent or technical literature in ever-increasing quantity. The annual reviews of "Petroleum Technology", published by the Institute of Petroleum, permitted a rapid appraisal of the state of knowledge in any particular aspect of the industry, but the increasing quantity of technical publications which had to be reviewed caused the editors to cease this most useful publication in 1952. No adequate replacement has yet appeared. Certain specific aspects of the subject have been collected in such works as the "Physical Chemistry of Hydrocarbons", edited by A. Farkas (1950), and the "Chemistry of Petroleum Hydrocarbons", edited by B. T. Brooks, C. E. Boord, S. S. Kurtz and L. Schmerling (1954). These contain chapters by a number of authors dealing with the state of knowledge of a particular aspect of the chemistry of hydrocarbons, but there is apparently no intention of publishing additional volumes which might serve to bring the subject-matter up to date.

A real need exists for this purpose, which has been recognized by the editors of the new publication, "Advances in Petroleum Chemistry and Refining". The editors state that the various aspects of the petroleum industry, economics and future trends, unit operations and design, refinery processes, petrochemicals and mechanical equipment, will be dealt with by specialist authors in separate chapters of a series of volumes which "over the course of several years will assume the character of a reference book". The two volumes under review constitute the first of this series, and contain a number of chapters devoted to each of the five group headings outlined previously. The individual authors are all active in research or development within the petroleum industry and many are already recognized as outstanding authorities on their subject.

The task of the individual authors has been simplified in that they are not bound by any restriction concerning the years to which their review must be devoted, nor apparently is it considered necessary to include a complete bibliography on the subject at the end of each chapter. Each author makes a critical appraisal of the state of knowledge in his particular subject at the time of writing and illustrates his comments by reference to such published work as he considers necessary for his purpose. In some instances, such as the excellent chapters on "Solvent Refining" (A. W. Francis and W. H. King) and on "Solid Polymers from Surface Catalysts" (H. N. Friedlander and W. Resnick), a fairly complete bibliography has been given, but in general the editors have not duplicated the bibliographies published annually in the Unit Process and Unit Operations review of *Industrial and Engineering Chemistry*. This permits a certain leisurely approach to the subject; the authors do not feel they have to cram a number of original references into every sentence but can deal at some length with the fewer references they choose to examine. The result is what our American friends might describe as an 'all time high' in technical review literature. The chapters are readable, and although not all of the same high standard are nevertheless interesting and in many cases stimulating.

Chemists and technologists in the petroleum industry will appreciate the critical treatment of the technical and patent literature by several of the authors, while the non-specialist will find much of general interest, particularly in the section dealing with economics and future trends. It is not often that books of this nature, intended for a specialized section of industry, can be recommended for general student reading, but in this case the books can be recommended to degree students and research workers in chemistry or chemical engineering. In particular, the chapter on "Solid Polymers from Surface Catalysts", by H. N. Friedlander and W. Resnick, is an excellent account of a rapidly developing field of chemistry which as yet has not been described in current text-books. Those interested in industrial developments will find the chapter dealing with "Raw Materials for the Petrochemical Industry", by P. W. Cornell and V. N. Hurd, of particular interest.

If the high standard of the first two volumes is maintained in future publications the series will prove a most welcome addition to the libraries of petroleum technologists, but it will only be welcome on these already overburdened shelves if it maintains its selective character and does not become a reference book.

F. Morton

CHEMICAL AND BIOLOGICAL ACTION OF RADIATIONS

Radiolyse de Liquides Organiques

Par Milton Burton. Polymérisations Amorcées par les Radiations Ionisantes. Par A. Chapiro et M. Magat. Effets des Rayonnements de Grande Énergie sur les Polymères. Par A. Charlesby. (Actions Chimiques et Biologiques des Radiations, Troisième Série. Collection dirigée par M. Haissinsky.) Pp. iv+222. (Paris: Masson et Cie., 1958.) Broché, 4,000 francs; cartonné toile, 4,800 francs.

Les Peroxydes Organiques en Radiobiologie

(Organic Peroxides in Radiobiology). Par R. Latarjet *et al.* (Actions Chimiques et Biologiques des Radiations, Quatrième Série. Collection dirigée par M. Haissinsky.) Pp. viii+156. (Paris: Masson et Cie., 1958.) Broché, 2,800 francs; cartonné toile, 3,600 francs.

In the third volume of Haissinsky's series of monographs the dominant theme, in each of the three topics discussed, is a physicochemical interpretation of the principal radiation effects. The various authors give at the outset a compact account of the theoretical principles which are used in the interpretation, and, in consequence, the articles serve as useful introductions to complex and expanding subjects. A further advantage of the approach is to focus attention on the studies which have led to results of a definitive character. It becomes apparent from the discussions that a great deal of published work in this field is of limited value, due to the fact that inadequate attention has been given to experimental conditions, such as purity of materials, methods of irradiation, and so on.

The theoretical background presented in the article by Burton is to some extent covered by Gray in the first volume of this series, but the reiteration, with greater emphasis on the role of excitation processes, and attention throughout to photochemical analogy, seems appropriate to the purpose in hand. His account of the radiolytic effects includes a range of organic liquids in various chemical classes, although the results obtained with hydrocarbons, which are the most susceptible to interpretation, occupy the major part of the discussion. The other two articles are authoritative and notably lucid. They will no doubt be read by radiobiologists in search of clues, but they also merit the attention of industrial polymer chemists.

The fourth volume is devoted to the proceedings of a colloquium concerned with an evaluation of the concept of organic peroxides as causal agents in radiobiological damage. Sixteen papers are presented, commencing with a useful account of recent work on the nature of the peroxides resulting from the autoxidation of fats. (The absence of literature references to this paper is an unfortunate omission.) The other topics range from the radiochemical production of peroxides in *in vitro* systems (cumene, polystyrene, nucleic acids), to the biological effects of peroxides, and the influence of oxygen on radiation effects produced in a number of biological systems. The discussions on the individual papers, and a valuable discussion on the main theme of the colloquium, are freely recorded in this volume.

The high standards set in the first two volumes of this series are well maintained in the present additions.

W. Anderson

SCIENTIFIC MEDICINE FOR POSTGRADUATES

Lectures on the Scientific Basis of Medicine

Vol. 7, 1957–58. (British Postgraduate Medical Federation: University of London.) Pp. xii+496+33 plates. (London: The Athlone Press, University of London, 1959.) Distributed by Constable and Co., Ltd. 45*s.* net.

Until a relatively short time ago, experience was the great postgraduate teacher, but the progress of knowledge in the past thirty years has made it clear that experience alone is no longer enough. Other teachers are necessary, capable of helping medical men and women to implement the new ideas and discoveries in their daily work whatever that may be. Twenty-three of these teachers have prepared their lectures for this book. It is the seventh in a well-known series, and the subjects covered are as wide as before. They range from studies of the growth of viruses to biochemical deficiencies in the whole animal, and include lectures on hæmatology, biochemical genetics, cell regeneration, pharmacology, minutiæ of the eye and ear, the stability of collagen and ageing processes in the female reproductive tract.

The subjects could only have been presented by an equal number of specialists, and they display the complexity of the biological framework as it is gradually becoming revealed to us. No one man can comprehend more than a fraction of it all in detail, but these lectures year by year are mostly simple enough to allow the interested reader to draw his own conclusions as to the bearing, if any, they may have on his daily round. Nevertheless, the spoken word is often easier to follow than the written one, and some of these lectures must be studied with care to get the most out of them, and for many people time and interest will be the limiting factors.

This series of books is described as a venture in postgraduate education, intended for graduates in medicine looking forward to careers as teachers and consultants in medicine and surgery and their special branches, as well as for those seeking careers in research and teaching in the medical sciences. This is a very fair appraisal and the sponsors may congratulate themselves on their venture. It is only one of the many now being made in postgraduate education. The short courses lasting from one to three days now being held at the Royal College of Physicians, at Cambridge and in many other parts of Britain, have rather a different appeal, but judging by their success, whether the proceedings are published afterwards or not, there is evidently a widespread demand for knowledge among specialists and practitioners, and an appreciation of the efforts which are being made to provide it for them.

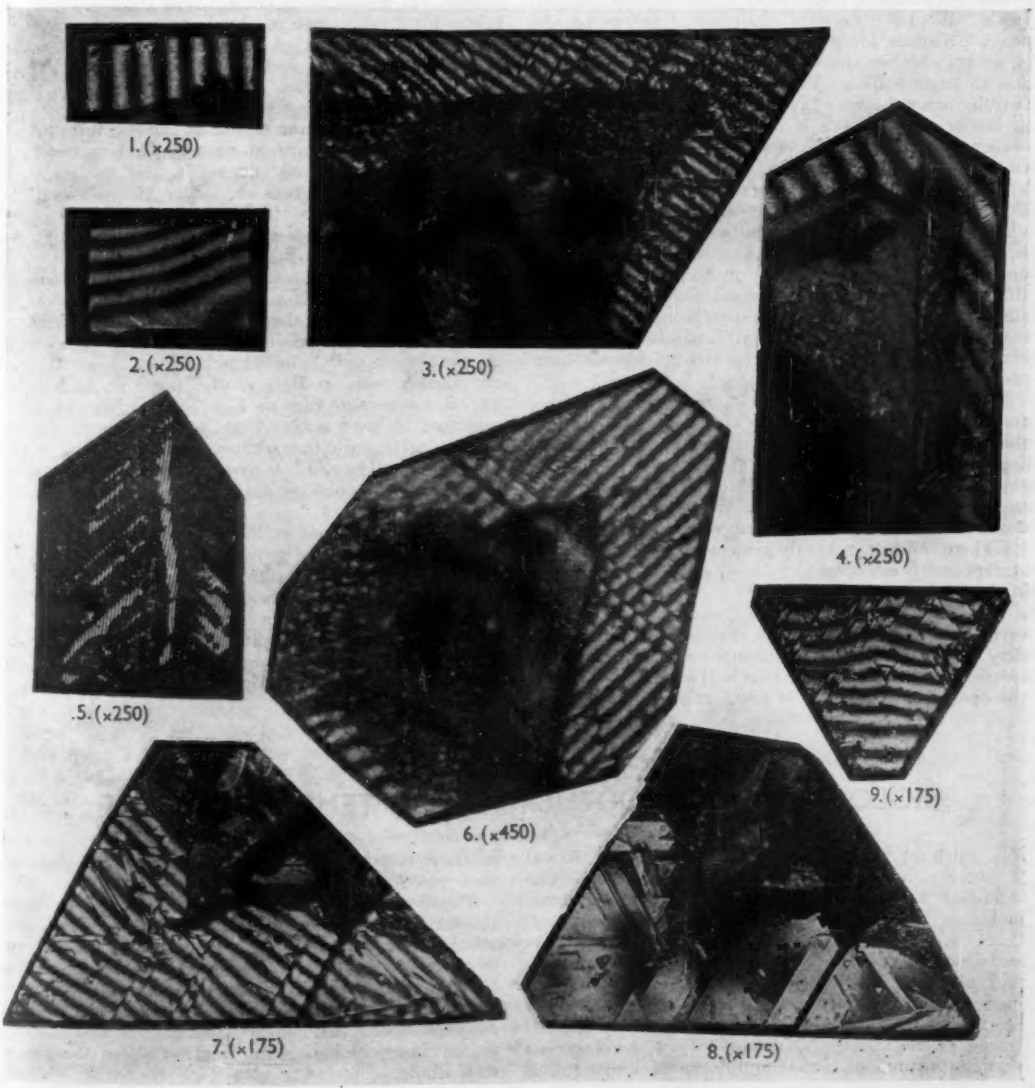
R. A. McCance

INTERFEROMETRIC STUDIES ON SYNTHETIC DIAMONDS

By Prof. S. TOLANSKY, F.R.S., and Dr. I. SUNAGAWA
Royal Holloway College, Egham, Surrey

In a recent article we reported on some findings on synthetic diamonds[1]. Using a very sensitive phase-contrast microscope we observed the following features which do not occur on natural diamonds, namely: (*a*) cubic faces of unusual smooth quality; (*b*) growth spirals on some such cubic faces; (*c*) dendritic diamonds; (*d*) very frequent linear slip lines, especially on octahedral faces (such slip is found in natural diamonds, but much less frequently).

We have now succeeded in obtaining interferogram on quite a large number of synthetic diamond faces. Owing to the micro-character and irregularity of shape of the material at our disposal, we have so far only succeeded with two-beam interferometry, but we are still pursuing attempts to apply multiple-beam methods. We have available both the samples reported upon previously and also a new sample (kindly sent to us by Dr. Bundy of the General Electric



Co., Schenectady). The older sample reveals many cubic as well as octahedral faces. The crystals in the second sample are perhaps twice as large linearly (some $\frac{1}{8}$ mm.) and are almost exclusively octahedra. Interferograms from the earlier sample will be considered first.

The interferograms were obtained by matching against glass. The fringe visibility found calls for comment. Normally the matching of gem-quality diamond against glass produces fringes of moderate visibility, because of the two very different reflecting coefficients. Here, on the contrary, many of the fringe patterns are of such excellent visibility that it must be concluded that the reflecting coefficients of the diamond surfaces are relatively low, indeed not very different from that of glass. This fringe visibility is particularly noticeable on some, but not all, of the fringe patterns, and it is quite possible that the few cases with poor visibility are due to a nearer approach in reflectivity to the typical natural value. We have no firm evidence as yet as to the possible causes for a reduction in reflectivity, that is, as to whether the defect lies in the crystal or is due to surface film. Figs. 1 and 2 show fringes on two fine smooth cubic faces. The smooth uniformity in the fringe patterns is ample confirmation of our earlier statement that synthetic cubic faces can at times show a remarkable degree of perfection. Fig. 1 shows an excellent plane (within the limitations of the two-beam fringes). The good fringe visibility is worthy of comment. Fig. 2 also shows a smooth, nearly flat, surface, but with a hillock over one corner, about one fringe in height. It is more than likely that this is an unresolved growth spiral, since we have most frequently seen such spirals starting out from corners. Two-beam interferometry cannot be expected to resolve the spirals we have previously described.

We consider next, in Fig. 3, an octahedral face, and this interferogram reveals three commonly found characteristics: (*a*) First is the marked skeletal or hopper character of the face. It reveals a level, fairly plane, parapet or rim and within this is a hollow region. (*b*) Second, the fringes on the rim or parapet show that this region is smooth and of good quality. (*c*) Third, and particularly noticeable, are the crystallographically oriented slip lines, some of which end in the surface.

We have found numerous frequently interesting variations of the hopper or skeletal formation and they are particularly well brought out by the fringe system, in that the upper rim is that which contacts the optical flat. Fig. 4 is one further example from many. There are local variations in fringe visibility here and it is well to remember that the good visibility comes probably from the defective regions, the bad visibility on the contrary being attributable to a more normal reflectivity. Indeed, there is a suspicion on the originals that the regions of good visibility of fringes are patchy and perhaps coated.

A few dendritic crystals have responded to interferometric analysis and part of one such dendrite is shown in Fig. 5. The fringes show that both the 'backbone' and the 'ribs' are flat and smooth, and indeed co-planar, more or less.

We come now to the consideration of the interferograms given by the second batch of crystals, largely octahedra. Here again the hopper character is common, as before, and the slip lines are frequent. Fig. 6, taken with blue light, reveals the hopper character and shows strongly marked slip running right across the crystal, even across the central hollow. It seems fairly certain that the slip here has taken place very late in the history of the crystal. By the employment of different wave-lengths the heights of the steps have been determined. They vary from 500 to 15,000 A.; a good deal more than might have been expected.

Fig. 7 shows a characteristic of the slip lines which we find to be very common, namely the way in which they terminate. Many do so in the surface, and the fringe displacements on either side show quite clearly that the slip step is inclined to the plane of the surface of the crystal. It has, in fact, the character of a screw dislocation.

Finally, we direct attention to the occasional appearance of the familiar growth trigons seen so often on natural octahedral faces. Their visibility, when shallow, is much enhanced by interferometry, in accordance with familiar principles. Thus trigons are clearly seen in Fig. 7, and more so in Fig. 8. Fig. 8 is the same face as Fig. 7; but here we have arranged to have a very high interferometric dispersion by setting the face almost exactly parallel to the optical flat. The result is to create the usual enhanced interferometric contrast and this shows up the trigons well.

The occurrence of well-defined linear trigons on diamonds which have grown very rapidly and which show not the slightest sign of etch or solution effects is in complete harmony with our long-held views that trigons are essentially a growth phenomenon. As so often observed in natural diamonds, so too in synthetic diamonds the trigons frequently 'sit' on a linear dislocation (Fig. 9).

[1] *Nature*, **184**, 1523 (1959).

BIOLOGY OF THE SOUTHERN COLD TEMPERATE ZONE

IN opening a discussion meeting of the Royal Society, held during December 10–11, which was attended by some twenty invited contributors, including many from overseas, the leader, Prof. C. F. A. Pantin, stressed the importance of understanding natural distribution patterns of animals and plants. The chief problem of to-day is not how to maintain a maximal rate of technological advance, but to predict and control the biological future of the Earth. The study of the development and adaptations of faunas and floras in the past, and of their response to climatic and geological factors, may provide vital information for the future.

The Southern Cold Temperate Zone is the least known of the world's major climatic belts, and information concerning it is widely scattered. Because of the wide separation of its land masses there has been a tendency for the separate consideration of their floras and faunas. This discussion was the first attempt during this century at a general synthesis of the available biological information about the region as a whole.

The Royal Society's interest in the Southern Temperate Zone arose in connexion with the Darwin–Wallace Centenary, which it celebrated by sending a five-man expedition to southern Chile, in the southern summer of 1958–59. This party carried out geological and ecological surveys, and made observations on species with a disjunct distribution linking Chile and New Zealand (M. W. Holdgate*). Originally the Royal Society had been interested simply in this trans-Pacific relationship; however, as a result of the expedition, it was realized that this should be considered within the broader context of the whole southern circumpolar region.

The Southern Cold Temperate Zone includes a large proportion of ocean: the climate of its land masses is therefore unusually closely related to marine temperatures and currents (G. E. R. Deacon). Southwards the Antarctic Convergence provides a sharp and fairly constant boundary: the land within it, like Antarctica itself, South Georgia and Bouvet Island, is largely ice-capped. Northwards, the Sub-Tropical Convergence fluctuates more widely but includes nearly all the temperate southern islands. Southern South America, New Zealand and Tasmania are the only truly continental southern temperate areas.

According to C. J. F. Skottsberg, many plant families must be regarded as Palæo–Antarctic, and are still largely confined to the southern cold temperate region (for example, Eucryphiaceae, Gunneraceae, Escaloniaceae, Proteaceae). Some members of these are strictly continental in distribution, but even those genera reaching oceanic islands fall roughly into either 'balanced', 'South American', 'Neozelandic–Australian' or 'Afro–Indian' groups. *Acaena*, widely spread around the zone, exemplifies the first type, *Pernettya* and *Fuchsia* the second, and *Hebe*, *Coprosma* and *Nothofagus* the third. Viewing the pattern as a whole, the paths of migration of these groups seem to radiate from the Antarctic. Their present distribution does not seem readily explicable in terms of trans-oceanic dispersal, and seems to accord best with an initial segregation in Antarctica itself and subsequent outward spread (C. J. F. Skottsberg and G. E. DuRietz). Such a hypothesis is strongly supported by geological evidence (R. A. Couper). In the late Cretaceous and early Tertiary *Nothofagus* species and Podocarpaceae were abundant in west Antarctica (Seymour Island), Australia, New Zealand, Kerguelen and southern South America. Many species disappeared from the southern areas in the late Pliocene, at which time a northward shift seems to have occurred. These plants have never been found as fossils in the northern hemisphere or the tropics, and their present extension into New Guinea and Indonesia is regarded as recent.

Vegetationally, southern Chile and New Zealand, as the two main southern temperate areas, have much in common (E. J. Godley). In both the wet, oceanic, western regions are dominated by evergreen rain forests in which *Nothofagus* species are prominent. The New Zealand forests are, however, much richer in species. Other differences include the lack of deciduous beeches in New Zealand (there are seven species in Chile), and the greater importance of podocarps as forest dominants there. Only the open 'Magellanic moorland' of south-western Chile is regarded as truly Sub-Antarctic. Bog vegetation of the 'carpet' type, formed by species of *Astelia*, *Donatia*, *Gaimardia*, *Oreobolus*, and similar plants, rather than *Sphagna*, prevails in this region and also in the New Zealand Sub-Antarctic islands, and is considered an important indicator of Sub-Antarctic conditions.

The southern islands also show fairly clear similarities in the structure of their vegetation. Only the northernmost (Tristan, Gough, Amsterdam, Juan Fernandez, Auckland, Campbell) have scrub, which is always of microphyllous prostrate evergreen trees bearing a heavy lichen and bryophyte epiphytic cover and with an under-storey rich in ferns (N. M. Wace). Large tussock-grasses (usually *Poa* spp.) form a characteristic formation on steep sub-maritime slopes throughout the zone. 'Wet heath' formations of Gramineae, Cyperaceae, ericoid shrubs and bryophytes form a higher belt, with areas of wind-eroded feldmark and montane grassland highest of all. These Sub-Antarctic lowland formations have a striking physiognomic resemblance to the vegetation of high tropical mountains (N. M. Wace and C. Troll); a fact which may be related to the small annual temperature variation in both regions (C. Troll).

In the Quaternary, southern Patagonia had at least three major glaciations. Pollen analysis reveals a post-glacial pattern of climatic and floristic change strikingly like that in the northern hemisphere (V. Auer). There is evidence that such processes are still going on, and the forest-steppe boundary in Tierra del Fuego seems to be fluctuating rapidly. Volcanic ash bands occur at constant horizons throughout most of Fuego-Patagonia, the three latest being dated at 9,000, 5,000 and 2,200 years before the present. These may perhaps be correlated with climatic changes (H. H. Lamb), and fluctuations in the position of the west wind belt and ocean currents are certainly to be expected to follow changes in temperature. A striking agreement in dating exists between the third Patagonian eruption and an ash band in the peats of Gough Island in the mid-Atlantic (U. Hafsten). However, pollen analyses of the Tristan–Gough group peats shows that the present mild oceanic climate, and the present vegetation, has persisted there for at least the past 5,000 years. The main vegetational changes seem to have followed human discovery (U. Hafsten).

The terrestrial fauna of the southern Chilean forests becomes progressively impoverished as one passes from the rich, warm-temperate, Valdivian type into the poorer Magellanic forests of the south, but the latter contain few species absent from the former (G. Kuschel). The western Magellanic moorlands are even poorer faunistically; on the other hand, the open, semi-arid steppes of eastern Patagonia are comparatively rich. Many southern Chilean invertebrate groups have a disjunct distribution in South America and Australasia, paralleling that of the plants, and some of the insects belong to orders which are strictly continental and known from no oceanic island (for example, Plecoptera, Megaloptera) (G. Kuschel and M. W. Holdgate). An outward migration from the Antarctic in the Mid-Tertiary may provide the best explanation of their present range. The terrestrial fauna of the southern oceanic islands, even of relatively warm examples like the Tristan–Gough group, is even more strikingly im-

* The names appearing in brackets in the text of this article are those of the contributors to the meeting whose papers are here summarized. It is hoped to publish all the major Contributions in the *Proceedings of the Royal Society* (B).

poverished. Only 132 terrestrial invertebrates have so far been recorded from these islands. Many of these show a strong South American affinity, and the insects are believed to have been derived predominantly from that continent by airborne immigration in the prevailing westerly winds (M. W. Holdgate). Occasionally South American Lepidoptera and land birds appear as stragglers in the islands. The Tristan group also shows slight faunistic affinity with St. Paul and Amsterdam, but none with the colder and more southerly oceanic islands. These do, however, share something of a truly circumpolar fauna with relations to South America and New Zealand. Most of these islands are believed to be young, and their faunas likewise. In the Tristan–Gough group this hypothesis is supported by botanical evidence (N. M. Wace).

Within the littoral marine fauna, it is possible to recognize a distinct southern circumpolar element paralleled in the algal flora (G. A. Knox). Many of the species are dominants and give a characteristic aspect to southern temperate shores. A schematic classification of these zonations can be derived, although distinct species groups are peculiar to each southern continent. Africa, despite an upwelling of cold Sub-Antarctic water off the south-west coast, has little truly southern cold-temperate faunistic relationship (T. A. Stephenson), a fact paralleled in its terrestrial invertebrate fauna (P. Brinck) and its flora (R. S. Adamson). In planktonic organisms, also, the southern waters have circumpolar, clearly zoned forms which show abrupt specific limitations near the convergences (N. A. Mackintosh). The Antarctic penguins also show somewhat of a circumpolar distribution with a radiation to distinct and geographically limited species farther north. In contrast, the shelf fauna of the Antarctic itself has a very high (70–80 per cent) incidence of endemism, and while many of its component species spread all around the continent they show no clear relationship with the animals of the other southern land masses (N. A. Mackintosh).

Oceanic birds are of profound ecological significance in the south, since they breed in vast numbers on many islands. One major vegetation type—tussock grassland—may be in part a biotically induced formation (N. M. Wace). Yet despite their abundance, these birds have so far been imperfectly studied and the validity of their taxonomic definition is still questionable in many cases (R. C. Murphy). Many species make great migrations, and may act as dispersal agents for seeds and invertebrates: this role may be filled especially by juvenile petrels, which often fly while still bearing down contaminated from the nest burrow (R. A. Falla).

There is no doubt that the Southern Cold Temperate Zone possesses many features as a whole. Despite wide separation, there are many floristic and faunistic similarities between southern America and New Zealand and Australasia, and this applies also to the structural features of almost all the southern cold-temperate vegetation types. There is geological evidence which accords with that of present distribution in suggesting a common origin for such groups in a temperate Antarctic during the Tertiary. In contrast, no terrestrial vertebrates have a distribution of the true southern cold-temperate type (P. J. Darlington), and indeed, except for birds, land vertebrates seem to be largely absent from the region. Those that do occur appear to be recent immigrants from the north. Ecological rather than distributional factors may underlie this anomaly. It is evident that many features of this zone reflect the exacting requirements of the high-oceanic climate. The species-poverty of the southern islands and of south-west Chile no doubt depends in part on dispersal difficulties and upon recent ice-retreat, but it may well also derive from the narrow and specialized range of habitat conditions. The Southern Cold Temperate Zone indeed emerges as an area with a highly characteristic biology, and with a climate more profoundly influenced by the temperature and circulation of the sea than any other major world zone (C. F. A. Pantin). It is apparent, however, that much more work remains to be done there. Native faunas and floras are fast vanishing before the onset of northern hemisphere species introduced by man; yet there is virtually no southern island or mainland area which can be said to have received adequate study in all respects.

M. W. Holdgate

AN INTERNATIONAL TAGGING TEST WITH SALMON (*SALMO SALAR* L.) SMOLTS

By A. SWAIN and P. E. CARADINE

Freshwater Fisheries Laboratory, Ministry of Agriculture, Fisheries and Food

FOR many years various experiments have been carried out in Great Britain and other European countries to investigate the migratory habits of the Atlantic salmon (*Salmo salar* L.). Much of the work has been undertaken to obtain further information about the movements of salmon in the sea, the proportion of smolts returning as adult fish and the percentage of spent fish surviving to spawn in following years, and in recent years thousands of salmon smolts have been tagged in Britain and Scandinavia.

Nearly all these experiments have involved the marking or tagging of the fish in some way. Numerous methods of tagging have been tried both with internal tags and external ones attached to various parts of the fish's body. The tagging of salmon smolts presents special difficulties because of the difference in the size of the fish between tagging and recapture. The lengths of most of the smolts tagged lie within the range 12–18 cm.; but when the fish are recaptured after spending one, two or more years at sea, the lengths will be between 50 and 100 cm.

or even longer. It follows that a tag which is attached to such a small fish must not restrict its freedom of movement unduly, but at the same time the tag must be such as to remain on the fish for a number of years. Also, in most instances, the tag must be clearly visible when the adult fish is caught. Although the type of tag has varied considerably, most of the workers have attached it near the base of the main dorsal fin of the fish.

In Great Britain, the number of tagged smolts being recaptured as adult fish has rarely exceeded 2 per cent. Norwegian returns have been somewhat lower, whereas in Sweden, the average figure has been between 5 and 10 per cent, with even higher figures for some of the larger smolts tagged. These high returns have been obtained both with Baltic salmon, where the fish are caught commercially on the feeding grounds, and with west-coast salmon, where they are caught along the coast near the mouths of the rivers.

The efficiency of different tagging methods was discussed at a symposium held by the Salmon and Trout Committee at the meeting of the Conseil International pour l'Exploration de la Mer, in October 1958. It was proposed that there should be an interchange of tagging methods. As a result, during April and May 1959, a number of Scottish smolts were tagged by Scottish, English and Swedish workers, using their own tags and techniques. Later on in May we visited Norway and Sweden in order to apply our English methods to the tagging of Scandinavian smolts.

All the smolts tagged in Norway and Sweden during this experiment were hatchery-reared and were two years old. We used two types of tag, one of which was a small silver plate, the other a small square of 'Ivorine' (celluloid)[1]. These tags were serially numbered, and for both types a short length of fine silver wire was used as a means of attachment. The Swedish tag (Carlin tag)[1] consisted of a small piece of cardboard coated with celluloid, with a message printed on one side of the cardboard. A serial number appeared on the other side. The tag is attached by means of a moveable hinge to a short length of soft stainless-steel wire which in turn is hinged to another stainless-steel wire, both ends of which are separately inserted into the body of the fish and twisted together on the other side. The tag used by the Norwegians is called the Lea hydrostatic tag[1] and is used in Britain for tagging adult fish. It consists of a small sealed plastic cylinder containing a printed message. It was attached to the fish by means of a bridle of soft stainless-steel wire. In all cases, hypodermic needles were used to facilitate the passage of the wires through the fish. A single needle was employed in the English and Norwegian techniques, a double needle in the Swedish method. The English and Norwegian workers attached the tag to the base of the anterior end of the main dorsal fin, so that the tag is carried on a loop above the back of the fish. The Swedish workers attached the tag to the body of the fish immediately below the dorsal fin. Thus, with the English and Norwegian methods, a small loop of wire extends over the back of the fish, whereas with the Swedish method there is no such loop. The Norwegian and Swedish workers anæsthetize the smolts with a 2 per cent solution of urethane for the tagging operation. The English workers do not normally use anæsthetics, but the fish are tagged in running water using a special holding block.

In Norway, more than two thousand smolts were tagged by the three different methods at the Lundamoen Hatchery near Trondheim. The fish were all released into the Gaula River. In Sweden, the production of electricity is based almost exclusively on the utilization of water-power, and a series of dams is being built on the main salmon rivers flowing into the Baltic Sea. The effect of these installations would be to destroy large areas of natural spawning grounds for salmon. The seriousness of such a situation would appear even more acute when it is realized that the particular value of the Baltic salmon fishery lies in its complementary relationship to the very important herring fishery in these waters. It has been decided, therefore, to substitute for the natural spawning grounds a system of hatcheries and rearing stations whereby the run of salmon can be maintained artificially by the annual release of a sufficient number of hatchery-reared smolts. In order to discover whether this system could be worked economically the Swedish authorities have tagged large numbers of artificially reared smolts during recent years.

A comparison of Swedish and English tagging methods was carried out at three stations on the east coast. The first batch of smolts was tagged at Galtström just south of Sundsvall. Fish reared at this particular station have normally produced a very high percentage of recaptures. The smolts were larger than normal, averaging 16–18 cm. in length and in excellent condition. More than four thousand smolts were tagged at Galström and all the fish were released into the nearby River Ljungan.

Another collection of smolts was tagged at Bergeforsen, near the mouth of the Indalsälven, one of the large rivers flowing into the Gulf of Bothnia north of Sundsvall. Here is situated one of the largest Atlantic salmon hatchery and rearing stations in the world. By means of a device consisting of batteries of plastic trays, more than two and a half million salmon eggs can be brought to the 'eyed' stage in quite a small room. The rearing station is constructed for the production of about four hundred thousand smolts of salmon and sea trout reared for two or three years. About 1,200 smolts were tagged at Bergeforsen and the fish were released into an artificial channel from which they could migrate into the main river at any time.

The third batch of smolts were tagged at Lulavan, situated on the Lule älv near the port of Luleå in Northern Sweden. The installation at Lulavan was merely a tagging station; but the history of the rearing of these smolts was most interesting. Although the salmon ova were from the north, the fish had been reared at Munka-Ljungby on the west coast of southern Sweden, where growth was more rapid. At the smolt stage, the fish had been transported back to the north by road tanker, a distance of 1,600 km. More than six hundred smolts were tagged at Lulavan and the fish were released into the main river.

In the spring of 1960, it is expected that Scottish, Swedish and Norwegian workers will tag smolts in an English river. Here, the fish will not be hatchery-reared but will be tagged during their natural migration out to sea.

It is hoped that some tagged fish will return in 1960; but it is clear that a statistical comparison of the methods of tagging employed in 1959 will not be possible until the end of the 1961 season, for only then will the major part of the 1959 smolt crop have been harvested.

[1] *J. Cons. Intern. Explor. Mer*, **9**, No. 2 (1953).

RESEARCH METHODOLOGY AT THE LONDON SCHOOL OF ECONOMICS

By Prof. M. G. KENDALL
Director of the Research Techniques Division

RESEARCH techniques in the social sciences grew up in a very haphazard fashion; and some of them are so rudimentary that they can scarcely be said to have developed at all. After the Second World War it was plain that, if effort was not to be misdirected and wasted, some systematic assault on problems of methodology in the social sciences was necessary. With the aid of a grant from the Nuffield Foundation the Research Techniques Division at the London School of Economics and Political Science was set up to study such problems. It has no precise terms of reference and ranges over a wide field; but, broadly speaking, it is concerned with the gathering and analysis of information and the testing of scientific hypotheses in economics, sociology, social psychology and related fields.

The Division began with a grant of £2,000 a year, a part-time director, a secretary and two research assistants. At the present time, having just celebrated its tenth birthday, it has a budget of about £30,000 a year and a staff, full-time or part-time, of more than thirty. These figures will probably increase by 50 per cent over the next two or three years. Most of the funds come from outside sources. Some of the staff are full-time research officers, others are teaching members of the School staff who are associated with particular projects and others again are outside consultants. The Division has its own computing unit and library.

Methodology of Social Surveys

The elicitation of social and economic facts is itself a complicated subject. It may involve the setting up of new systems of scale or measurement unknown to the physical sciences. Even the 'mere' collection of data involves extensive methodological study, especially in the field of social surveys. From the planning of a survey, through the design of the questionnaire, the selection of the sample, the handling of the interview situation, the coding of responses and the final analysis, there lies a long chain of procedure which can introduce error at almost any point along its length. The Division carries out studies of the nature and extent of such errors in order to devise methods of reducing them and of validating the final results. It has, for example, published studies of the sampling problems involved, of the effect of using amateur interviewers, of coding errors and of the way in which response varies with the type of question asked.

More recent studies embarked upon cover the adequacy of mail questionnaires, memory bias in respondents and the characteristics of persons who volunteer for panels. On the sampling side, although the drawing of samples of human beings in the United Kingdom is now fairly well understood, there remains a number of intractable problems for study: the sampling of rare events, the sampling of firms or retail shops, and, overseas, the sampling of migrant populations or under-developed areas.

Dynamic Economics

Many natural scientists who have taken their interest in economics so far as to read a text-book must have wondered whether it is not possible to reduce the subject to the much more formal lines with which they are familiar in their own discipline. It is not, I think, true that economics can be 'turned into a science' in the sense that the methods of the natural sciences can be taken over unchanged and applied in a new field. But I believe it to be true (and this belief forms the starting-point of the Division's work on economics) that with suitable new techniques or adaptations of old ones the behaviour of economic systems is capable of far more exact study than has been attempted in the past. Under the inspiration of Prof. A. W. Phillips, and with the help of a substantial grant from the Ford Foundation, the Division is engaged in a large-scale attack on this problem.

The first requirement is to bring the postulated relationships of economics to an explicit form for quantitative study—to write down the equations. This is itself far from easy. The second problem is to write down the model of the system and to investigate its behaviour. The third stage is to fit such models to real life, which involves intricate problems of estimation and testing of hypotheses. The final stage is then to use such models in the regulation of economic systems.

It is particularly interesting that the main stimulation for such work has been furnished by electrical engineers, who have realized the formal similarity between economic systems and the feed-back networks of control engineering. The economic problem, of course, is more complicated, especially in regard to the amount of random variation and unknown time-lags to which economic systems are subject. The Division has been working in this field for several years, and has now reached the point where most of the economic relationships can be formulated, the estimation problems solved and the numerical computations carried out on an electric computer. The properties of artificial models are now being studied in preparation for the next phase, which is the study of actual portions of the economy.

Operational Research

Operational research—the science of making the best of limited resources in complex man–machine situations—is a comparatively new subject in both emergence and scope. With the aid of a small grant from the Department of Scientific and Industrial Research, the Division has spent two years in self-education, preparing bibliographies and running a fortnightly seminar attended by operational research workers from outside the School. It is now proceeding with research studies on some of the methodological problems involved in such topics as linear programming and queueing theory, and members of the Division are engaged in actual operational research

for certain firms. One collateral development has been the setting up of systematic courses at the London School of Economics for postgraduates drawn from industry. It is by now quite plain that this side of the Division's work will expand substantially in the years to come.

Research on Statistical Method

A great many problems coming to the Division's attention are necessarily of a statistical character, and attempts are made to solve such theoretical statistical problems as arise. The work covers a very large field. At the mathematical end, for example, it has included the tabulation of the distribution of the characteristic roots of a random-dispersion matrix. A considerable number of studies have been published on distribution theory, estimation and inference, the theory of statistical relationship, time-series and stochastic processes. Such work, though in the main theoretical in character, usually stems from problems of analysing complicated and imperfect data such as arise in multivariate systems.

Bibliographical Work

A great deal of work within the Division's sphere of interest, as in scientific fields generally, is concerned with ascertaining what is being done elsewhere. A number of individual bibliographies have been published, for example, on queueing theory and on the life testing of industrial equipment. Some special studies have also been undertaken. For example, a "Dictionary of Statistical Terms" has been prepared on behalf of the International Institute of Statistics and Unesco; a new *Journal of Statistical Abstracts* has been founded; and the Division is on the point of going to press with the first volume of a comprehensive bibliography of papers on statistical method and probability from the sixteenth century up to the end of 1958 which will ultimately comprise about 25,000 titles.

Team Work

It is a characteristic feature of much of the Division's work that its problems require collaboration between workers in several different fields. Problems of survey research, for example, need mathematicians and statisticians at the sampling stage; psychologists at the interview stage; numerical analysts and statisticians at the summarizing stage; and sociologists or economists at the interpretative stage. Dynamic economics requires—in addition to economists—electrical engineers, numerical analysts and mathematicians. The members of the Division are, in consequence, drawn from all sorts of disciplines, and it frequently calls on the wide resources of the teaching staff at the London School of Economics as well as on those who are formally attached to it. The consequent interactions have done much to contribute to the Division's development and, I hope I may say, to its successes.

OBITUARIES

Sir Henry Tizard, G.C.B., A.F.C., F.R.S.

It is often said that you cannot keep a good man down, but you only have to look at the early history of many eminent people to realize how much they owe to chance. Tizard was thirty-five before he took the step which enabled him to develop his powers to the full. I believe that it was then that he took the quite conscious decision to become a V.I.P. He certainly achieved his greatness by his own efforts.

Tizard's forebears were all connected with the sea in some way or another and he, himself, was destined for the Navy, but failed because of eyesight. His mathematics gained him an exhibition at Westminster School, and at the outbreak of the First World War he was a Fellow of Oriel College, Oxford, having got there by way of a first-class degree in natural sciences at Magdalen, a year at Berlin under Nernst and two years at the Davy Faraday Laboratory of the Royal Institution.

When the War actually broke out, he was in Australia attending the meeting of the British Association. He returned as quickly as he could and joined the Royal Garrison Artillery, where he got himself into some difficulties owing to his highly unorthodox methods of training recruits. This, in fact, was not really his 'cup of tea' at all and he quickly succeeded in getting transferred to the Royal Flying Corps. By 1917 he was captain-in-charge of all experiments on aeroplanes at Martlesham Heath and was afterwards appointed as deputy to Bertram Hopkinson at the headquarters of the Ministry of Munitions. He carried on after Hopkinson had been killed in August 1918 until he was demobilized in the spring of 1919, when he returned to Oxford.

His experiences during the War had led him to realize the immense importance of the application of science to military affairs. He decided that he would never be outstanding as a pure scientist, and Oxford, therefore, did not give him the scope that he needed to develop his talents. Towards the end of 1920 he joined the Department of Scientific and Industrial Research, and in 1926, at the age of forty-one, became secretary of the Department in succession to Sir Frank Heath. In the same year, he was elected a Fellow of the Royal Society.

In 1929 he was appointed rector of the Imperial College of Science and Technology—a post which he held for thirteen years. He did, in fact, a very great deal for the College, but, during this period, he did a great deal more in other directions.

Ever since the First World War, Tizard had maintained his interest in aeronautics. He had been chairman of the Royal Aeronautical Society in 1924, and in 1933 he was appointed chairman of the Aeronautical Research Committee—a post that he held for ten vitally important years. However, the work for which I shall always give Tizard the highest marks in that period was the job that he did as chairman of the Committee for the Scientific Survey of Air Defence which started work at the beginning of 1935. Tizard's colleagues on this body were originally A. V. Hill, P. M. S. Blackett and H. E. Wimperis, with A. P. Rowe as secretary. I shall always believe that this body, and particularly Tizard himself, were the people who made it possible for Watson-Watt to develop radar, which was the

biggest contribution to the winning of the Second World War. You cannot read the accounts of the development of radar written by such varying authorities as A. P. Rowe, Lord Swinton and Watson-Watt himself without coming to the conclusion that it was Tizard's drive from the outside which ensured that radar was developed at such rapid speed—and not only developed, but also deployed in a usable form.

As the War progressed, Tizard served in the Ministry of Aircraft Production and also as the first —and last—external civilian member of the Air Council. In August 1940 he led a British mission to the United States with the object of disclosing all our scientific secrets. He took with him in his pocket a sample of the magnetron—the thermionic device, perfected in Britain by Randall and Boot, which really made radar work. One American historian said of this visit: "When the members of the Tizard mission brought the magnetron to America in 1940, they carried the most valuable cargo ever brought to our shores. It started the whole development of micro-wave radar and constituted the most important item in reverse lease-lend". In the years after the War, when information on American military research was hard to obtain, Tizard was not slow to recall his war-time mission.

In 1942 Tizard resigned from the Ministry of Aircraft Production and the Air Council to become president of Magdalen College, Oxford; but, although he now had no formal Government appointment, he still continued to advise informally on various scientific problems, and in 1943 he went out to Australia to advise the Australian Government. It was during this trip that he bearded General MacArthur in his den and shattered the morale of the General's aides by flatly refusing to regard him as anything but an equal. Incidentally, he also won a case of sherry off the General by a bet on the length of the War.

By 1946 Tizard was sixty-one. He was very comfortably ensconced as president of Magdalen and it looked as though the excitements were over. His greatest work was, in fact, still to come.

One of the major lessons of the Second World War was the vital necessity to apply science to all forms of Government activity, and this could only really be done provided the right type of scientist was brought into full collaboration in both the formation and implementation of policy. The Services learned this lesson very thoroughly indeed during the War and, as a result, the old Deputy Chiefs of Staff Committee was replaced by a new body in the Ministry of Defence known as the Defence Research Policy Committee. The Committee consisted of those responsible in the Service Departments and the Ministry of Supply, both from the operational and from the scientific angle, for the formulation of requirements for new weapons and for research and development. Its chairman was to be a scientist of high standing appointed for a period of years. At the same time, on the Civil side of Government, a committee known as the Advisory Council on Scientific Policy was also set up. Tizard was persuaded to assume the chairmanship of both these bodies; and, although from 1946 he served full-time in the Ministry of Defence, he gave a great deal of attention to the work of the Civil Council.

Between 1946 and 1950 Tizard laid the real foundations of co-operation at the top level between the scientist and the military authorities. His own position and effectiveness did much for the recognition in other fields of the need for, and value of, scientific advice. I do not believe there was any other person who could have done this with anything like comparable success. For one thing, in spite of the fact that neither he nor anybody else regarded him as an outstanding pure scientist, his standing in the scientific world was extremely high. Even by 1946, five universities had awarded him honorary degrees, and in subsequent years five more followed suit. For another, he had shown, in the days before the Second World War, his capacity to see what was important, from the national point of view, to develop and, perhaps even more, the ability to get other people in high places to accept his view. He inspired—and in this he was almost unique among the leading scientists of the day—immense respect and confidence among officers of all the Fighting Services. He had a very genuine appreciation of, and sympathy with, their problems and they all knew he wanted to help.

He dominated any committee of which he was chairman, not—as so many other people try to do—by talking all the time, but by his skilful appreciation of the characteristics of the people sitting with him and of the appropriate moment at which to intervene. Sometimes, these interventions took the form of an exhibition of bad temper. Most people thought this was spontaneous; but, in point of fact, it was most carefully rehearsed, as those who had to brief him for the meeting well knew. In those days, his moments of real anger were quite rare, and they almost invariably arose out of his unceasing warfare with what he regarded as administrative stupidity.

He was, perhaps, at his most impressive when presiding over the periodic meetings of the Commonwealth Advisory Committee on Defence Science, to which scientists and serving officers came from all over the Commonwealth. Here, he inspired a slightly awed but affectionate respect. He was, of course, a brilliant conversationalist, and the entertainments which accompanied these Commonwealth meetings gave him opportunities which he seized to the full.

He was on excellent terms with the Chiefs of Staff, particularly in the earlier days. As time went on, however, he began to be regarded as the 'Great Man', and his visits to the Chiefs' meetings became rather more formal occasions. There was, in fact, something rather magisterial about his manner, especially on any formal occasion. Even on an informal occasion, he was not a man with whom one took undue liberties. Nevertheless, to those who worked with him closely, he was a very real friend and by no means difficult.

In 1950 his health began to fail and by doctor's orders he had to slacken off a great deal. He did not take kindly to this change, but there was no doubt that he began to get tired rather quickly and then became a little bad-tempered. He recognized this, however, and I can only remember one occasion which had unfortunate results. By 1952, Tizard had begun to lose some of his confidence in himself. He thought that his health had deteriorated to such an extent that his useful working life was over, and he therefore resigned from the public service. There is no doubt that a measure of the old zest and vitality had gone out of him.

He was not, however, a man who could be idle, and for the remainder of his life he occupied himself

as a director of Glaxo Laboratories, Ltd., and other public companies. In spite of many attractive offers, he refused to join any commercial organization primarily concerned with the Defence effort. He took the view—and I am sure rightly—that he had been in far too central a position in the defence field to allow him to make his special knowledge available to a single company engaged in such work.

His health improved appreciably after he retired, and right up to the day of his death he was the most delightful and inspiriting dinner companion. A typical form of activity into which he entered with all his usual enthusiasm was the year he spent in 1955–56 as Prime Warden of the Goldsmiths' Company. I am sure the Company benefited from his wisdom.

No account of Tizard would be complete without some reference to his quarrel with Cherwell. This originated in 1936 over the Committee on the Scientific Survey of Air Defence, and eventually got so bad that the two men could not endure in silence each other's presence in the same city. This, indeed, was the only matter on which I ever found Tizard really unreasonable, but Cherwell was worse. In fact, in this matter, both these eminent gentlemen behaved like a couple of spoiled children. The tragedy of it was that this quarrel prevented Tizard gaining the confidence of Winston Churchill when the latter returned to power in 1950.

By any standards, Tizard was a great man who made a large contribution to the nation's affairs. He had a very real foresight and was rarely proved wrong. I am still waiting for someone to take seriously the warning which he issued towards the close of his presidential address to the British Association in 1948. He knew little about agriculture in detail, but, as with all the other subjects he touched, he had a wide view of the necessities. "I shall predict", he said, "that unless the prevention of disease among plants and animals and all other scientific problems of the supply of food are studied on the same kind of scale by men of similar calibre as are the problems of human health, chaos and misery will result". How right he was.

F. BRUNDRETT

Dr. Mary D. Waller

DR. MARY DÉSIRÉE WALLER, who died at the age of seventy-three on December 11, was widely known for her precise and beautiful work on vibrating plates and as an outstanding teacher of physics. She came of distinguished scientific ancestry, her father being Augustus Désiré Waller, who first showed that the electric currents set up by the beating of the human heart could be recorded, and her grandfather Augustus Volney Waller, whose name is commemorated in 'Wallerian degeneration'.

Educated at Cheltenham and Bedford College for Women, as a young woman she was appointed demonstrator in the Physics Department of the London (Royal Free Hospital) School of Medicine for Women, a Department of which she later became head and in which she continued her research after her retirement in 1947. It was in 1932 that she first came into prominence by her discovery that metal plates could be thrown into regular vibration by the suitable application of a block of high-density carbon dioxide. It shows great scientific percipience that her discovery derived from the fact that an itinerant vendor of ice-cream had directed her attention to the way in which a bicycle bell rattled when in contact with ordinary solid carbon dioxide.

With this solid carbon dioxide technique, the mode of action of which she explained, she carried out extensive and precise studies of the modes of vibration of metal plates of square, rectangular, circular and elliptical form, illustrated by beautiful photographs, the series recording the vibrations of a square plate being particularly striking. Her last published work included some interesting observations on the ridges formed by particles of different size and shape in the sound field between a vibrating plate and a parallel horizontal surface. At the time of her death she had nearly completed a book dealing with her work on vibrating plates, the appearance of which has been announced by the publisher. It is to be hoped that a colleague will finish the work and see it through the press.

Dr. Waller was a woman of great integrity and kindliness, whose brightness and charm made her a welcome figure in scientific, and other, circles. As an accomplished teacher she endeared herself to generations of students in her Department. She was devoted to the subject that she made her own, but her interests were wide and she was a delightful conversationalist. She lived for many years with her friend, Dr. Bertha Turner, at a charming house in the neighbourhood of Regent's Park, in which she died.

E. N. DA C. ANDRADE

Mr. Cyril L. Collenette

CYRIL COLLENETTE, who died suddenly on November 2 at the age of seventy-one, devoted most of his life to the study and service of natural history. His boyhood days were spent in the stimulating surroundings at Woodford, then a country parish in the heart of Epping Forest, and this early bent for the pursuit of Nature's secrets and offerings was never to leave him. He became a world-wide traveller naturalist in such places as Malaya, French Guinea, the Matto Grasso and British Somaliland, making valuable additions to collections, especially of insects.

Collenette's main life's work began and ended at the British Museum (Natural History), South Kensington, as an associate and world authority on the large family of moths, the Lymantridae. Apart from his museum work, he was a keen spare-time botanist and bird observer, ever willing to pass on his experiences and recordings to such bodies as the British Trust for Ornithology (he was one of its earliest ringers), and the London Natural History Society (a member since 1907 and one-time president). He was on the Committee for the Birds of the Royal Parks, as well as on that of the London Natural History Society during the compilation of its book, "Birds of the London Area".

His own books include "A History of Richmond Park", written during 1937 when living at Richmond, and "Sea-girt Jungles", written on his return from a long Pacific sea voyage on the *St. George*, a sailing vessel which visited such places as the Marquesas, Easter Island and the Galapagos. He told me that this was one of the most exciting and rewarding episodes of his natural history career.

His colleagues at South Kensington will miss their tall and friendly companion, and all who sought out his advice and friendship will mourn an ever-willing helpmate and kindly friend.

ALFRED LEUTSCHER

NEWS and VIEWS

Vice-chancellor of the University of Monash: Prof. J. A. L. Matheson

Prof. J. A. L. Matheson sailed for Australia before Christmas to take up his appointment as vice-chancellor of the University of Monash, Melbourne, and to begin his exciting and responsible task of building a new University. Matheson, who was educated at Bootham School, York, succeeded the late A. H. Gibson as Beyer professor of engineering in the University of Manchester in 1951, having been lecturer in civil engineering in the University of Birmingham from 1938 to 1946 and professor of civil engineering in the University of Melbourne from 1947 to 1950. During his tenure of the Beyer chair at Manchester, Matheson re-organized the engineering courses and planned new laboratories to house the post-war expansion. In addition, he played an important part in the wider field of university building and planning. He directed research into various aspects of theory of structures, including the use of scale-models and the load-carrying capacity of rigidly jointed triangulated steel frames. It was largely due to his guidance that so much has been done in Manchester to further the use of electronic digital computers for the analysis of complex structural problems. His work culminated earlier in the year in the publication of his book, "Hyperstatic Structures", which is impressive for its scope and scholarship.

Civil Engineering at Manchester: Prof. M. R. Horne

Dr. M. R. Horne, lecturer in the Department of Engineering, University of Cambridge, has been appointed professor of civil engineering in the University of Manchester and will take up the post next summer. Dr. Horne was born on December 29, 1921, and was educated at Boston Grammar School and Leeds Grammar School before going to St. John's College, Cambridge, in 1939. He had a distinguished academic career, obtaining honours and the Archibald Denny Prize in the Mechanical Sciences Tripos, 1941. He was awarded the John Winbolt Prize for an essay on an engineering subject in 1944. During 1941–45 Horne worked for the River Great Ouse Catchment Board as an assistant to the District Engineer, Bedford, surveying and carrying out reconstruction of weirs and sluices. He returned to the Department of Engineering, Cambridge, in 1945, joining the team which was then being assembled to continue the work, started before the War in Bristol, on the plastic behaviour of steel structures. He obtained the Ph.D. degree in 1950. In 1951 he was appointed an assistant director of research on the introduction of the postgraduate course of instruction on structures and materials, the first such course to be introduced by the Cambridge Engineering Department. Horne transferred to the post of University lecturer in 1957, in which year he was elected a Fellow of St. John's College. In the fifteen years that Horne has been back in Cambridge he has played a prominent part in the development of the theory of the plastic behaviour of structures and of the design method based on it.

Director of the Forest Products Research Laboratory: Dr. F. Y. Henderson, C.B.E.

Dr. F. Y. Henderson, who retires from the directorship of the Forest Products Research Laboratory, Princes Risborough, in October, was reader in timber technology at the Imperial College of Science and Technology. Initially interested in plant physiology, he took over Percy Groom's Sub-department of Technology of Woods and Fibres; the elementary text-book on wood technology which he wrote then proved specially popular with those in the timber trades. Henderson's tenure of the directorship marks a period in the history of the Forest Products Research Laboratory. He took over in 1945, when many relatively unknown woods were beginning to replace well-tried ones, and these needed investigation and assessment in relation to the traditional timbers; new and little-known insect and fungal pests claimed attention; plywood had developed enormously and the Laboratory played its part in research on such laminated wood. This work, and other aspects, like wood bending, machining and adhesives, might have been mentioned—it was Henderson's task to co-ordinate and guide. Moreover, the past few years have seen the enormous expansion of co-operative work with the Forestry Commission and the beginning of research on the pulping of home-grown timbers. The steady flow of important bulletins from the Laboratory emphasizes the volume of varied work carried out there since the War and, during this time, trade and private inquiries have occupied much of the time of the staff. Henderson's retirement marks the end, more or less, of this period. Relieved of most of the inquiry work, his colleagues have more time for the fundamental work for which the laboratory is well equipped. Moreover, reconstituted wood products, like hardboards and laminated wood, are replacing solid timber in many of its traditional uses and presenting their own special problems to the Laboratory.

Mr. H. Wooldridge, O.B.E.

Mr. Henry Wooldridge, who will succeed Dr. Henderson, has been a member since its formation of the Steering Committee of the Forest Products Research Laboratory. He has spent most of his career at the headquarters of the Department of Scientific and Industrial Research, where he is at present head of the Stations Division, and as such is responsible for liaison with all the Department's laboratories. Previously, he was deputy establishment officer in the Department, and before that he served two secretaries of the Department, Sir Frank Smith and Sir Edward Appleton, as private secretary. Mr. Wooldridge joined the Department in 1935 from the British Association for the Advancement of Science, of which he had been assistant secretary for five years. He was educated at Reading School and King's College, London.

Royal Society and Nuffield Foundation Commonwealth Bursaries

Awards under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have

been made as follows: Dr. R. N. Brothers, senior lecturer in petrology, University of Auckland, to enable him to examine at the Imperial College, London, the application of crystalline and deformation theories for certain New Zealand ultrabasic rocks to a group of related rocks which occur in Great Britain, for a year from November 1959; Dr. J. W. Clark-Lewis, senior lecturer in organic chemistry, University of Adelaide, to enable him to continue a study of the chemistry of natural products, with particular reference to the stereochemistry of flavan derivatives, at the Imperial College, London, for seven months from October 1960; Dr. A. W. Crompton, director of the South African Museum, Cape Town, to enable him to study mammalian palæontology at Cambridge and the British Museum (Natural History) for four months during 1960; Dr. J. C. Gould, senior lecturer in bacteriology, University of Edinburgh, to enable him to extend his studies of staphylococcal infections and make comparative studies in an African community, in Nigeria, during May–September 1960; Prof. K. R. L. Hall, professor of psychology, University of Bristol, to enable him to carry out a field-study of behaviour and social organization of wild baboon groups in differing environments in South and South-West Africa, during July–September 1960; Dr. L. M. Jackman, lecturer in organic chemistry, Imperial College, London, to enable him to investigate hydrogen bonding in organic molecules by nuclear magnetic resonance spectroscopy, at Adelaide, during July–October 1960; Prof. D. O. Jordan, Angas professor of physical and inorganic chemistry, University of Adelaide, to assist him to visit University College, London, and the Royal Cancer Hospital, London, during February–September 1960, in order to study new techniques and particularly those concerned with the structure, behaviour and functioning of biological macro-molecules; Prof. H. Kleerekoper, professor of zoology, McMaster University, to assist him in completing during the summer of 1960 an investigation of bottom sediment cores of Lake Moneague, Jamaica, for which a bursary was awarded to him in April 1958; Mrs. Beryl E. March, instructor and research associate, Department of Poultry Science, University of British Columbia, to assist her to visit Cambridge during May–August 1960 in order to study methods of evaluating the nutritive value of fish meals; Dr. E. H. Mercer, of the Chester Beatty Research Institute, London, to enable him to carry out an electron microscopic study of cell division and differentiation in plants, at Sydney, during February–July 1960.

The Liverpool Geological Society

THROUGHOUT the first three days of 1960 the centenary celebrations of the Liverpool Geological Society, happily combined with the eighth Inter-University Geological Congress, attracted an enthusiastic gathering of more than two hundred geologists. The theme of the meetings was "Recent Advances in Geology", and each of ten lectures by speakers from British and foreign universities gave rise to spirited discussion. The Society has now published the text of these addresses in a volume of more than three hundred pages which is a fine contribution to geological literature (*Liverpool and Manchester Geol. J., Centenary Issue:* price 20*s*.). In it, T. S. Westoll reviews recent studies on the palæontology of fishes: G. Voll, in a sixty-page paper, summarizes the latest work in the field of petrofabrics; S. I. Tomkieff describes sources of information on the geology of the U.S.S.R.; L. M. J. U. van Straaten writes on deltaic sedimentation; R. G. Mason on geophysical investigations of the sea floor; W. S. Mackenzie on experimental studies in petrology; W. C. Krumbein on numerical data in geology; C. F. Davidson on radiogeology and geochronology; and R. H. Cummings on advances in micropalæontology. An essay by W. E. Swinton tells the history of *Chirotherium*, the reptilian foot-print found in the Triassic rocks of Cheshire (long believed to be the hand-print of a mammal) which is reproduced on the seal and medal of the Society. Liverpool geologists have done well in sponsoring this readable review of present knowledge in some of the most active branches of earth science.

Uranium in South-West England

THE reconnaissance survey for uranium, carried out by the Atomic Energy Division of the Geological Survey during the past two years, is now virtually complete. Seventy new occurrences of uranium have been found during the course of operations which have included an aerial survey of Cornwall, Devon and much of Somerset, as well as surface mapping, excavating and drilling at promising localities. No deposit of economic importance has been discovered, and it is concluded that if any are present they are covered to such a depth with soil or overburden that they are unlikely to be detected by existing instruments. Although no deposit estimated to contain more than 5 tons of uranium oxide has been found as a result of this survey, the South-West of England uranium province has served for many years as an ideal testing ground for British geological electronic equipment, which is now used extensively throughout the world. The study of uranium occurrences in Cornwall and Devon, started in 1945, has played an important part in the discovery of uranium in the Commonwealth, while recent work has contributed considerably to the development of aeroradiometric surveying techniques.

Scientists and Engineers in American Industry

THE U.S. National Science Foundation has stated that American business firms employed about 780,000 scientists and engineers in January 1959. About 35 per cent of the total were engaged in engineering and scientific research and development activities. Scientists and engineers in the industry sectors included in the current survey are estimated to represent approximately two-thirds of the scientists and engineers employed in the United States. The other third are employed in government agencies, educational institutions, non-profit organizations, and self-employed. Industries employing the largest groups of scientists and engineers in January 1959 were aircraft and parts, electrical equipment, and chemicals and allied products. Each employed more than 75,000 scientists and engineers; combined, they employed one-third of the total number of scientists and engineers in private industry and approximately half the scientists and engineers engaged in the performance or administration of research-and-development activities in private industry. Each of two other industries employed more than 50,000 but less than 75,000 scientists and engineers: machinery (except electrical) and engineering and architectural services. Next in size, in terms of scientific and engineering employment, were construction, fabricated metal products and ordnance,

primary metals, and motor-vehicles and equipment; each of these industry groups employed more than 30,000 but less than 50,000 scientists and engineers. Employment of scientists and engineers in private industry was about 4 per cent higher in January 1959 than in January 1958. Engineering employment increased from about 605,000 in January 1958 to 630,000 in January 1959. The number of scientists employed in private industry increased from about 145,000 in January 1958 to 150,000 in January 1959.

British Digital Computer Information Centre

The National Centre of Information on Automatic Programming of Digital Computers has been established by the Department of Mathematics of the Brighton Technical College in response to a recommendation of the first National Conference on Automatic Programming held in Brighton in April 1959. The purpose of the Centre is: to establish and maintain a comprehensive library of publications; to publish, in conjunction with Pergamon Press, Ltd., an annual review of automatic programming; to provide a 'clearing house' for information and inquiries on automatic programming; to organize small working conferences on particular aspects of the subject; and to maintain permanent contact with organizations in all other countries concerned with such matters. An advisory committee to assist the work of the Centre has been established and includes Dr. A. D. Booth (University of London), Mr. R. A. Brooker (University of Manchester), Mr. P. V. Ellis (International Computers and Tabulators, Ltd.), Dr. S. Gill (Ferranti, Ltd.), Dr. J. Howlett (United Kingdom Atomic Energy Authority), Dr. R. J. Ord-Smith (Standard Telephones and Cables, Ltd.), Mr. C. Robinson (English Electric Co., Ltd.), Mr. Christopher Strachey, Mr. A. E. Taylor (Remington Rand, Univac), Mr. J. H. Wensley (Computer Developments, Ltd.), and Mr. M. Woodger (Mathematics Division, National Physical Laboratory), together with the following as corresponding members: Dr. Grace Hopper (Remington Rand, U.S.A.), Dr. L. Luckaszewicz (Mathematical Machines Laboratory, Polish Academy of Sciences), and Prof. H. Rutishauer (Institute of Applied Mathematics, Zurich). Further information can be obtained from the organizing secretary, Mr. Richard Goodman, Department of Mathematics, Brighton Technical College.

Hospital Morbidity Statistics in Great Britain

A further contribution to the study of morbidity has recently been published by the General Register Office (The Registrar General's Statistical Review of England and Wales for the Year 1955. Supplement on Hospital In-Patient Statistics. Pp. vii+206. London: H.M. Stationery Office, 1959. 10*s.* 6*d.*). This volume summarizes the results of the hospital in-patient inquiry for 1955. This inquiry, which is steadily becoming more comprehensive, is expected to cover virtually the whole of the discharges of in-patients from hospitals in Britain by 1957; but in 1955 the hospitals that participated did so on a voluntary basis. Nevertheless the principal specialities seemed to be represented in close correspondence with the national figures, and the inquiry was fully representative of two hospital regions, East Anglia and Wales, for which special statistics are given. The statistical unit used in the inquiry is the discharge of an in-patient from a hospital, and the principal tables in the volume show the number of such discharges in various age and sex groups, and in different regions, by principal condition diagnosed. The number of deaths in hospital is shown separately, and there are data dealing with the duration of stay and the waiting period before admission to hospital. Special tabulations deal with children in hospital and maternity cases. Teaching and non-teaching hospitals are distinguished throughout. While hospital morbidity statistics are not a wholly reliable guide to morbidity, they can, as the report indicates, be of considerable assistance in the study of the mass aspects of disease, and the data will also be of considerable value to the hospital administrator. It is to be hoped that future tabulations will give information about the socio-economic status of the patients discharged. The appearance of this volume provides a valuable addition to our knowledge of the functioning of the National Health Service.

Transvaal Museum

After a lapse of four years it is fortunate that it was possible once again, during July 1959, to publish the *Bulletin of the Transvaal Museum*. A topical article on "Excavating Ape-Men", by Mr. J. T. Robinson, describes the cave limestone in which such remains are found. The grid system of excavation is described and all bone is treated with 'Glyptal' cement immediately after it is removed from the breccia. Afterwards a 10 per cent solution of acetic acid is used to free the bones from the surrounding matrix.

Musical Rites

Certain hard igneous rocks ring when struck and the note obtained depends on the dimensions of the rock itself. By careful shaping different notes can be obtained and a sort of 'glockenspiel' created. There is a decorated rock-shelter in Nigeria where such a 'glockenspiel' occurs to which parties come to be married, their faces being first carefully whitened. Dr. B. D. Malan describes just such a rock gong site in the Parys district of the Orange Free State in the *South African Journal of Science* (**55**, No. 2). It is well known that music plays a prominent part in the lives of many primitive folk; these gongs are very interesting and the resulting 'music' not at all unpleasant.

The Commonwealth Scholarship Commission

The Earl of Scarbrough has been appointed chairman of the Commonwealth Scholarship Commission in the United Kingdom. The Commission is to be set up under the Commonwealth Scholarships Act, 1959, recently passed by Parliament, to administer the Commonwealth Scholarship and Fellowship Plan in Britain. This Plan was worked out by the Commonwealth Education Conference at Oxford in July 1959 and creates a thousand new postgraduate awards throughout the Commonwealth. Of these, the United Kingdom will contribute five hundred scholarships and fellowships tenable in the United Kingdom at any one time.

University News: London

Dr. G. V. R. Born, research officer at the Nuffield Institute for Medical Research and departmental demonstrator in the Department of Pharmacology in the University of Oxford, has been appointed to the Vandervell chair of pharmacology tenable at the

Institute of Basic Medical Sciences. Dr. D. L. J. Bilbey, assistant professor of anatomy in the University of Toronto, and Dr. R. M. H. McMinn, lecturer in the University of Sheffield, have been appointed to readerships in anatomy tenable at King's College.

The title of professor of economics in the University of London has been conferred on Mr. B. S. Yamey in respect of his post at the London School of Economics and Political Science.

The title of reader in physics in the University of London has been conferred on Dr. D. M. MacKay in respect of his post at King's College.

Southampton

THE Institution of Structural Engineers is to provide funds for the establishment of a readership in structural engineering in the Department of Civil Engineering of the University. The Institution will make available £2,000 per annum for seven years at the end of which period the Council will be responsible for the appointment.

Borough Polytechnic, London

THE following appointments in the Borough Polytechnic have been made: G. Isserlis, senior lecturer in charge of the Division of Metal Science; Dr. D. B. Smith, senior lecturer in food technology; S. N. Houldsworth, lecturer in chemical engineering; Dr. B. E. Weller, lecturer in physical chemistry; Dr. H. W. Hykin, lecturer in mechanical engineering.

The Night Sky in February

FULL moon occurs on Feb. 12d. 17h. 24m. U.T., and new moon on Feb. 26d. 18h. 24m. The following conjunctions with the Moon take place: Feb. 6d. 16h., Aldebaran 0·5° S.; Feb. 22d. 00h., Jupiter 5° S.; Feb. 23d. 04h., Saturn 4° S.; Feb. 24d. 07h., Mars 5° S.; Feb. 24d. 13h., Venus 4° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Saturn on Feb. 7d. 11h., Venus being 0·2° N., and Venus with Mars on Feb. 17d. 03h., Venus being 1·1° N. Mercury can be seen as an evening star during the later part of the month, setting at 19h. 15m. on February 25. It reaches greatest eastern elongation on February 23. Venus is a morning star, rising about 2 hr. before the Sun at the beginning of the month. Later in the month it becomes too close to the Sun for observation. Mars is badly placed for observation, rising about an hour before the Sun. Jupiter is a morning star, rising at 5h. 00m., 4h. 20m. and 3h. 35m. on February 1, 15 and 29, respectively. It may be seen low in the south-east before sunrise; its stellar magnitude is −1·5. Saturn is also a morning star, but it is still too close to the Sun for satisfactory observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Feb. 2d. 19h. 49·3m., μ Psc. (*D*); Feb. 6d. 14h. 26·0m., Aldebaran (*D*); Feb. 6d. 15h. 10·0m., Aldebaran (*R*); *D* and *R* refer to disappearance and reappearance, respectively.

"Salicylate and Potassium Fluxes of Rat Diaphragm"

WE regret that certain printing errors appeared in the communication by Dr. J. A. Hicklin, published in *Nature* of Dec. 26, p. 2029. In the penultimate line of the second paragraph, the word 'active' should be deleted. In the third paragraph, the second sentence should read: "Contrary to expectation the principal effect of salicylate was consistently to accelerate the *efflux* of potassium-42", and the fourth sentence of the paragraph refers to estimates of *influx*.

Announcements

THE Medical Research Council has recently established the following new research units: the Blood Coagulation Research Unit, by agreement with the United Oxford Hospitals, at the Churchill Hospital, Oxford, under the part-time direction of Dr. R. G. Macfarlane; and the Environmental Radiation Research Group under the honorary direction of Prof. F. W. Spiers in the Department of Medical Physics, University of Leeds.

FOLLOWING the appointment of Captain R. A. Villiers as the first director of the Scientific Instrument Manufacturers' Association of Great Britain as from October 1, 1959, it has been announced that Mr. G. L. Knight has been appointed assistant director. Mr. Knight will operate under Captain Villiers in the field of information and external activities, and will be responsible for the collection and distribution of information for the Association.

A SMALL travelling exhibit on rocket astronomy has been lent to the Science Museum, London, until the end of February by the United States Naval Headquarters. Pictures show the launching of rockets and photographs of the Sun taken from the rockets. An actual camera which has been recovered after use in a rocket is included in the exhibit, which is explained by a recorded talking commentary.

THE Institute of Electrical Engineers has announced that the Faraday Lecture will be given by Prof. M. G. Say on February 17, at the Central Hall, Westminster, and will be entitled "Electrical Machines". Further information can be obtained from the Secretary of the Institute of Electrical Engineers, Savoy Place, London, W.C.2. Admission, which will be by ticket only, is free.

AN all-day meeting of the Welsh Soils Discussion Group will be held on February 3 at University College, Aberystwyth. The programme will be introduced by Mr. J. R. Thom, director of forestry for Wales, and succeeding papers will deal with forestry and soils. Further information can be obtained from Mr. J. A. Taylor, Geography Department, University College, Aberystwyth.

A SYMPOSIUM on "Basic Questions in the Structure of Languages" will be jointly sponsored by the American Mathematical Society and the Association for Symbolic Logic at the Hotel New Yorker in New York during April 14–16. At the same time and place the American Mathematical Society is also organizing a two-day symposium on "Stability Problems in Hydrodynamics". Further information can be obtained for both symposia from the Associate Secretary, American Mathematical Society, 190 Hope Street, Providence 6, Rhode Island.

A COURSE of ten lectures entitled "Reactor Fuel Element Technology" will be given in the Department of Chemical Engineering at the Imperial College of Science and Technology on Thursday afternoons during February 11–March 17. This course is designed to lay emphasis on the industrial problems of reactor fuel-element technology. The speakers will be from the United Kingdom Atomic Energy Authority. Further information can be obtained from the Registrar, Imperial College of Science and Technology, London, S.W.7.

CENTENARY OF "THE ORIGIN OF SPECIES"

SOME BOTANICAL ASPECTS

THE Conference arranged by the Botanical Society of the British Isles to celebrate the centenary of the publication of the "Origin of Species" was held during November 27–29 and was well attended by members and visitors.

The President, in his introductory remarks, directed attention to the importance of Darwin's friendship with Hooker in the development of the botanical arguments in the "Origin".

Lady Barlow enlarged on this theme, among others, in the course of a most interesting and illuminating account of her grandfather's botanical activities. She pointed out that, though Darwin had benefited greatly from his taxonomic work on Cirripedia, his work on plants was entirely of an experimental kind and laid the foundations of a great deal of the subsequent development of these branches of botany.

Prof. T. W. Böcher (Institute of Plant-anatomy and Cytology, Copenhagen) traced the development of cytotaxonomy since Darwin's time. He pointed out that though Robert Brown discovered the nucleus when Darwin was twenty-two, and when he was only thirty-nine Hofmeister discovered chromosomes, what is now called cytotaxonomy was not born until this century. He regarded Rosenberg, who in 1909 demonstrated a polyploid series in *Drosera*, as the Father of this branch of botany. Rosenberg had many distinguished pupils, among them Täckholm and Heilborn, who together with Jørgensen, Tischler, Winge and, in Britain, Ruggles Gates, Blackburn and Heslop-Harrison placed the study on a firm footing in the 1920's. Among others who played an equally important part in this early development were Clausen, Hagerup, Langlet, Percival and Senjaninova. Meanwhile, in the U.S.S.R. under Navashin another school grew up more concerned with ideograms and karyotypes than with chromosome numbers.

Both these approaches, combined with the discovery of apomixis, permanent structural hybridity and allopolyploidy by later workers, have been extremely fruitful.

Not least among the benefits which botanists have derived from the growth of cytotaxonomy is the kind of information which should deter them from, as Prof. Böcher said, "attempting species-making in groups where any cutting up into described taxa will, at most, have only local or temporary importance".

The vital necessity of combining careful taxonomic work with cytological studies was pointed out and illustrated by reference to *Campanula rotundifolia* and *Triglochin maritima*, among others. *C. rotundifolia* is a variable complex consisting of rather local diploids, usually clearly distinguished from one another but resembling tetraploids growing in the same neighbourhood, which are themselves widespread and intergrading. The variation here seems to be mostly due to structural hybridity and has so far defied satisfactory taxonomic analysis. In the *Triglochin maritima* complex, on the other hand, there is a polyploid series ranging from $2n$ to $24n$ and at least some of the members of this series have distinct morphological characters and distributions, and correspond to previously described though not generally accepted species.

After surveying recent work on genera and families, Prof. Böcher pointed out that the names cytotaxonomy, experimental taxonomy, biosystematics or synthetic taxonomy are perhaps superfluous. It might be better just to use the word taxonomy, "presupposing that all taxonomists now and in the future to the best of their ability are cyto-geno-eco-choro-taxonomists".

Prof. W. H. Camp (Connecticut) in the course of an illuminating and entertaining hour described some of the ways in which evolution is occurring in the world to-day, illustrating his lecture with examples from his own experience. During the past century biologists had paid little more than lip-service to Darwin, and it was only recently that we had obtained a real insight into the mechanism of evolution.

His own approach to the problem had been *via* population genetics, which attempts, to begin with, to discard the classical species-concept and to study either populations in the field or samples of them in cultivation. From studies of this kind series of group concepts develop which are often, though not necessarily, identical with the traditional species, and at the same time a great deal of information is obtained about evolutionary processes which are going on now and often also about those which have taken place in the past. Knowledge of the cytology and breeding systems of the plants with which he is concerned, as well as the 'taxonomic eye', are essential for the accurate interpretation of the biosystematist's observations.

The variation in morphology and ecology in American species of *Fagus* and *Vaccinium* can be explained largely by the effects of Pleistocene changes in climate which gave the opportunity for introgression between a number of distinct but homoploid species, followed by selection for particular combinations of physiological and morphological characters adapted to the various habitats available in post-glacial times. Corroborative evidence for this is provided by the fossil remains of *Fagus* in various parts of the North Temperate regions and by the variation in kind and quantity of alkaloids in the bark of different *Cinchona* populations. In the *Vaccinium corymbosum* complex the situation is further complicated by the production of both auto- and allopolyploids.

Prof. Camp went on to demonstrate the extremely rapid changes which occur in *Viola* populations in response to variations in light-intensity, humidity and other habitat factors. This is a group in which morphological and physiological variation is very great and sterility barriers almost non-existent.

Attempts at subdivision into species seem to be meaningless as there is a continual evolution of new genotypes from chasmogamous flowers and, at the same time, the abundant production of seed from cleistogamous flowers allows of rapid increase of the best-adapted individuals in a population.

In conclusion, he suggested that evolution is perhaps best regarded not primarily as the production

of new species but as the genetic ability of organisms to fill the multitudinous new habitats being constantly presented to them by the changing environment. Species may then be considered as 'ephemeral nodes in the genetic reticulum' within the space-time continuum.

Prof. D. H. Valentine (Durham) spoke about evolution in *Primula* section Vernales in which there are nine species, all with $2n = 22$, and all but two, *P. veris* and *P. lofthousei*, capable of crossing with fairly good meiotic pairing in the hybrids. Most species have long peduncles and erect capsules with dry seeds but in three with short peduncles the capsules lie on the ground. Of these *P. vulgaris* has sticky ant-dispersed seeds and occurs over quite a wide area, but the other two, with dry seeds, are very local endemics, the difference being perhaps attributable to the relative efficiency of the dispersal mechanisms.

It is possible to arrange the species of this section in a series based on the ease with which they can be crossed. This does not altogether agree with the morphological sequence, and, in the absence of almost all knowledge of the genetic mechanism and physiological basis of 'crossability', it is impossible to be certain which is nearer the phylogenetic truth. It is clear that evolution in this group has been gradual and continuous and is combined with geographical and ecological isolation rather than sterility barriers. In other sections of the genus, for example Farinosae, polyploidy has played an important part.

Dr. N. Hylander (Uppsala) gave an account of some trends in modern research work on Scandinavian plants. He pointed out that a number of species previously unknown in Scandinavia had been discovered, even in the past decade, a remarkable thing in a region which has for long possessed an unusually high concentration of botanists. The possibility of perglacial survival has been the subject of many investigations, and, Dr. Hylander thought, had perhaps been rather overdone. So many species have been added to the list of survivors that the supposed nunataks now gave the impression of having been overpopulated. Endemism in the *Papaver radicatum* group, he suggested, might after all be of relatively recent origin. The ecotype concept of Turesson is an important Scandinavian contribution to botany and one which, combined with cytological work, has occupied the attention of many workers.

Variation within agamospecies has been observed in a number of plants and the basis of it investigated by Sørensen and Gudjonsson in triploids of the *Vulgata* group of *Taraxacum*. Here it has been shown that loss of one, or rarely two, chromosomes is responsible for most of the variations, and that *gigas* forms arise through polyploidy. Detailed study of the taxonomy and the method and speed of dispersal of microspecies has yielded much information about plant migrations and the age of different floristic elements.

Prof. I. Manton (Leeds), before proceeding to discuss some aspects of evolution in Pteridophyta, said how much she owed to the stimulation and instruction she had received from both Rosenberg and Prof. W. H. Lang. She then outlined some of the main aspects of the cytology of the group, pointing out that, since high basic numbers and polyploidy were frequent, it was important for cytologists to be well acquainted with the twenty-nine times table. In some tropical species of *Ophioglossum* diploid numbers greater than 1,000 occur and it is difficult to imagine the mechanism by which pairing is achieved at meiosis.

Allopolyploidy appears to be the chief means by which species have been formed, at least among Polypodiaceae in the broad sense. About 50 per cent of British ferns are known to be allopolyploids, one parent of which is often known; the other parent is sometimes to be found in N. America and most of those at present unknown will probably be found in temperate Asia when that region is better explored botanically.

From a fairly exhaustive study of the cytology of Pteridophyta in Britain, Madeira, Ceylon, Malaya and Tropical Africa it is evident that in this group polyploidy is not a response to cold, as has been suggested for angiosperms. In Ceylon and Tropical Africa 60 per cent of the species so far investigated are polyploids.

Natural hybrids occur among the ferns far more frequently than is generally recognized, but the F_1 generation is often so like one parent that they pass unnoticed. Prof. Manton estimated the number of living species of ferns at approximately ten thousand and between them at the very least five thousand hybrids occur. It is thus evident that ample opportunities exist for continued evolution through allopolyploidy.

Dr. G. D. H. Bell (Plant Breeding Institute, Cambridge) demonstrated how 'pure' cytotaxonomy was being applied to the improvement of crop plants. He made it clear that the highest taxonomic skill combined with a good knowledge of cytology and genetics, and adequate facilities, were essential for this work and that so far, even with *Triticum*, no more than a small beginning had been made.

The collection of wild relatives of crop plants from centres of genetic variation followed by a thorough study of the genetics, cytology and taxonomy of the material provide the basic information, which in *Triticum* has led to a good understanding of the genomes of the cultivated hexaploids. New amphidiploids produced, with the aid of colchicine, from crosses between *Aegilops ovata* and *6n* wheats have enabled increased genetic variability to be introduced into this important crop plant. Resistance to *Erysiphe graminis*, for example, can be brought in from the *Aegilops* parent and promises to be valuable. A technique has recently been worked out for introducing one or two *Secale* chromosomes into hexaploid wheat through suitable hybridizations, thus giving an entirely new source of variation which may prove of practical importance.

Dr. Bell pointed out that not only was taxonomy *sensu* Böcher the basis of plant-breeding but also that the benefits were reciprocal; indeed, work of this kind could go far towards solving the problem of world food supplies.

An interesting series of exhibits illustrating recent research on the subjects of Darwin's botanical works was on view and directed attention to the variety of lines in which he was the pioneer. It is perhaps not generally known, for example, that he was the first person to use coleoptiles in the experimental investigation of phototropism.

A highly successful and enjoyable meeting concluded with a conversazione and a visit to Down House.

A full account of the Conference will be published by the Society.

T. G. Tutin

UNIVERSITY STUDENTS AND THEIR ACADEMIC ORIGINS

WITHIN the past ten years there have been several valuable studies of student performance at universities in the United Kingdom, some concerned with 'wastage', or failure to complete a course of study; some with degree of success achieved by those who did not fall by the wayside; and some (of which the survey under notice is an example) with both. A variety of factors make it difficult to compare the results of such investigations. First, they usually relate to one particular university or college, where conditions affecting initial selection of students and their subsequent academic progress may differ materially from those obtaining elsewhere. Secondly, they are often based on the intake of a particular year (differing from the year chosen in other surveys), and year-to-year fluctuations in the quality of students are believed to be considerable, at least within one college, faculty or department. Thirdly, to serve the purpose of the inquiry, success or failure is analysed in terms of a limited number of characteristics, which vary from one survey to another.

The limitations imposed by these and other factors are well brought out if the survey relating to the Queen's University of Belfast (An Audit of Academic Performance: a Study of the Prediction of Academic Success in the Queen's University of Belfast. By Michael Forster. Pp. 75. Belfast: Queen's University, 1959. 7*s*. 6*d*.) is put side-by-side with another very recent one, undertaken by the Cambridge University Sociological Society (Academic Careers of Entry of 1955). There is no need to emphasize the importance of the different setting provided by the Queen's University of Belfast as compared with the University of Cambridge for a study of academic performance. In the Queen's University case the students concerned are those who entered in the four-year period 1946–49; for Cambridge, the intake of one session, 1955–56, was taken. For the purpose of the Belfast inquiry, degree of academic success was analysed in terms of four main possible associated factors: performance in the Northern Ireland Senior Certificate Examination (the main mode of entry), age at entry, whether educated at a Belfast or a non-Belfast school, and whether a scholarship holder or not. In the Cambridge inquiry the factors selected were two in number. The first was type of secondary school attended, and here the main classification separated Headmasters' Conference, Direct Grant, Grammar and other schools from each other (but did not, unfortunately, distinguish between predominantly boarding and predominantly day schools within these categories). The second analysis involved comparing the performance of entrance scholars and exhibitioners with that of commoners.

Although few comparisons can usefully be made between two studies with such very different settings and purposes, this does not, of course, imply that they were not worth undertaking. Their value lies, to begin with, in providing a factual basis for discussion of the topics with which, individually, they were designed to deal. Is the examination, on the basis of which entrance to the Queen's University is largely determined, a satisfactory index of probable future performance? Are Cambridge colleges admitting too high a proportion of entrants from certain types of school, at the expense, perhaps, of other entrants more likely to show good academic results? Another obvious value of these surveys is that, when further investigations are made in the same universities for later years, it will be possible to identify some of the changes that have taken place. Again, each new survey makes some contribution to the methodology of work in this particular field. Finally, where a number of inquiries relate to the intake of different universities in the same year, there is at least the possibility of fruitful inter-university comparison. The Cambridge survey, for example, has taken the year used in the study of admissions undertaken by me for the Committee of Vice-Chancellors and Principals, and this is also the basis on which Prof. D. V. Glass and his colleagues are conducting a large-scale follow-up inquiry which should throw considerable further light on the social factors influencing academic success among students at British universities.

R. K. Kelsall

PLANT–ANIMAL FOOD CYCLES IN THE SEA

MICROSCOPIC phytoplankton floating passively in the sea have proved a suitable field for the techniques of population dynamics. Gordon Riley and others have shown that when populations of plants, and also the herbivores that graze on them, are considered in terms of their content of organic carbon, it is possible to predict roughly the varying quantities in different areas in terms of the changes in the physical environment. The problem, however, from the point of view of fisheries studies, is that only one part of this generalized organic matter may be relevant. Thus, herring will feed on copepods but not on salps, both of which graze on the phytoplankton. In this way an area of high primary production is a necessary but not a sufficient condition for a commercial fishery.

A paper by Dr. D. H. Cushing, of the Fishery Laboratory at Lowestoft, is of interest because in a study of the organic production in the southern North Sea he has introduced details of the species of plants and animals involved ("On the Nature of Production in the Sea". Fish Invest., Lond., Ser. II, Vol. 22, No. 6. Pp. iii+40. London: H.M. Stationery Office, 1959. 10*s*. net). In place of the abstract idea of quantities of animal carbon eating quantities of plant carbon, he has developed an 'encounter' theory

of grazing relating the speed of movement of copepods and the density of plant cells. This gives grazing-rates which are realistic in terms of the experimentally determined food requirements of the animals and the rates of depletion of plants in natural environments. His other innovation is to compute the growth of the *Calanus* populations, which are the dominant herbivores in the area studied, in terms of: (1) relations between plant density and egg laying; (2) the times taken to pass through the naupliar and copepodite stages; (3) probable mortalities at the various stages. His theoretical model of the production during the spring outburst of phytoplankton then gives results in terms of the numbers of *Calanus* (and similarly other copepods) that will be present at different times. The usefulness of this analysis has been shown in Dr. Cushing's previous work, where his results suggested that the aggregation of herring shoals on *Calanus* patches and their subsequent disengagement depended on a threshold concentration of *Calanus* in the patches ("Production and a Pelagic Fishery." Fish. Invest., Lond. Ser. II, Vol. 18, No. 7. Pp. 112. London: H.M. Stationery Office, 1955. 12*s*. 6*d*. net).

These detailed advantages, however, have certain drawbacks. Analyses in terms of particular species do not permit the easy comparison with other areas which is an important feature of the more abstract methods. For example, by conversion from plant cell-volume, Dr. Cushing gets a value for basic production during the spring. This value is only about one-quarter of those usually calculated for comparable areas, but it is difficult to see reasons for this divergence.

Another disadvantage of Dr. Cushing's model is that the possible effects on the plants of low nutrient concentrations are not included. Theoretically, this factor is used to make the more 'conventional' models stable; practically, it allows them to deal not only with the spring outburst when nutrients are comparatively plentiful, but also with summer conditions when they are scarce. Dr. Cushing points out, however, that although nutrient supply finally determines the quantity of production, this supply may depend not merely on the rate of uptake by the plants but also on the rate of return of inorganic nutrients to the water through herbivore excretion—a factor about which we know far too little. This could be particularly important in tropical oceanic areas where nutrients are at very low concentrations and where the high water temperatures may accelerate the biological re-cycling. Dr. Cushing's suggestion that production in these areas may be higher than formerly supposed is borne out by recent evidence, given at the International Oceanographic Congress in New York in September by Menzel and Ryther, that a significant outburst of production can occur in the northern Sargasso when nutrients are extremely low. Thus, production may depend on a balance between the physical and biological rates at which nutrients are made available to the plants. In the richer temperate and arctic areas, production may be mainly controlled by the supply of nutrients from the deeper waters, but it is possible that in tropical areas the rate of production is largely independent of such features of the physical environment and depends on how fast the plant-herbivore communities can re-cycle their nutrients.

Dr. Cushing, by his individual approach to these questions, has shown up some of the failings of the abstract methods, and particularly the need to make use of information about species composition and behaviour.

J. H. STEELE

IMMUNITY OF MICE SURVIVING SYSTEMIC LEUKÆMIA (*L*1210) TO ANTIFOLIC RESISTANT VARIANTS OF THE DISEASE

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TWO of the halogenated derivatives of amethopterin, 3′5′-dichloroamethopterin and 3′-bromo-5′-chloroamethopterin, have been demonstrated to be markedly effective in the treatment of the transplantable acute lymphoid leukæmia *L*1210 in mice[1–4]. Employing these derivatives of amethopterin, it was possible to obtain extensive survival time and an appreciable number of survivors of systemic leukæmia *L*1210, even when treatment was initiated only several days prior to anticipated day of death.

With a relatively high level of inoculum (about 1,000,000 cells/mouse) injected subcutaneously, the *CDBA* mice employed in the above studies (*BALB/c An* × *DBA/2J*)F_1 male mice succumbed characteristically in 10–12 days with systemic leukæmia, and a local tumour at the site of leukæmic inoculation (10–12 mm. in diameter). On treatment of advanced leukæmia with the dihalogenated derivatives of amethopterin, the local tumour at the site of leukæmic inoculation usually regressed after several treatments, and survival times of 90 days and beyond were not uncommon[4].

The mice that survived systemic leukæmia following treatment with the halogenated derivatives of amethopterin showed immunity on reinoculation of the leukæmia[4,5]. Many of the mice appeared to be highly refractory, and the leukæmic re-inoculum showed no evidence of growth. Other mice showed partial immunity. In the latter case, the re-inoculum grew slowly, but progressively, and the animals

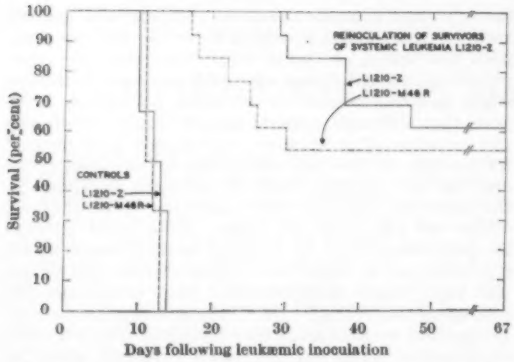

Fig. 1. Inoculation of the sensitive leukæmia (*L*1210*Z*) and an amethopterin-resistant subline (*L*1210–*M*46*R*) into *CDBA* mice surviving systemic leukæmia *L*1210 following treatment with 3′5′-dichloroamethopterin and 3′-bromo-5′-chloroamethopterin. Following treatment of leukæmic (*L*1210*Z*) mice with 3′5′-dichloroamethopterin and with 3′-bromo-5′-chloroamethopterin from day 7–90 there were 45/290 survivors on day 160. The mice were re-inoculated with *L*1210*Z* at that time, and there were 26/45 survivors on day 218 (ref. 5). These 26 mice were employed in the current study. Re-inoculated animals, 13 per group; controls, 6 mice per group, including the animals given the experimental and 1/10 of the experimental inoculum. Day 0 equals day 218 following the initial inoculation of leukæmia *L*1210. *L*1210*Z* Gen. 242; *L*1210–*M*46*R* Gen. 56. Approximately 500,000 cells per mouse, subcutaneously

eventually died, showing a rather extensive increase in survival time as compared with controls.

Similarly, the immune response to leukæmia *L*1210 has been elicited in 'out of strain' mice without the need for therapy[5]. When leukæmia *L*1210 was introduced subcutaneously into *BALB/c An* mice it grew at the site of inoculation. In some instances the growth was progressive, resulting in the death of the *BALB/c An* mice. In most instances the tumour regressed following the initial growth, and the mice were immune to re-inoculation of the disease.

The current study was undertaken to determine whether *CDBA* mice, apparently cured and immune to leukæmia *L*1210 as the result of therapy with the halogenated derivatives of amethopterin, or *BALB/c* mice, made immune to leukæmia *L*1210 following spontaneous regression of the tumour, would also be immune to antifolic resistant variant sub-lines of leukæmia *L*1210. For this study two antifolic resistant variants were employed: (*a*) An amethopterin-resistant variant of leukæmia *L*1210 (*L*1210–*M*46*R*). This variant of leukæmia *L*1210 appeared in response to therapy with amethopterin over several tumour generations[6]. *L*1210–*M*46*R* is resistant to therapy with amethopterin and shows a high degree of cross-resistance to the halogenated derivatives of amethopterin[6]. (*b*) The variant *L*1210–*M*66–3*R* was developed by therapy with 3′5′-dichloroamethopterin in serial transfer of leukæmia *L*1210. It has been demonstrated to be resistant to treatment with 3′5′-dichloroamethopterin over a wide range of doses[7].

The results of re-inoculation of drug-treated *CDBA* survivors of systemic leukæmia (*L*1210) with leukæmia *L*1210 and the resistant variant *L*1210–*M*46*R* are shown in Fig. 1. Treatment with 3′5′-dichloroamethopterin and 3′-bromo-5′-chloroamethopterin had been administered daily from the 7–90 day following leukæmic inoculation and the second inoculation with leukæmia *L*1210*Z* of the survivors had been on day 160[4,5]. The third inoculation (Fig. 1) employing *L*1210*Z* and *L*1210–*M*46*R* was on day 218. Fig. 1 shows that the mice with systemic leukæmia *L*1210, rendered immune by extensive treatment with the halogenated derivatives 3′5′-dichloroamethopterin and 3′-bromo-5′-chloroamethopterin, were immune, not only to the inoculation of the *L*1210 sensitive tumour, but were also immune to the *M*46*R* resistant variant of leukæmia *L*1210.

With both the *L*1210*Z* and the amethopterin-resistant variant *M*46*R* there were a considerable number of animals alive at 67 days following inoculation (285 days since the first inoculation of the leukæmic cells) (Fig. 1). At 67 days there were 8 out of 13 mice alive that had been re-inoculated with the sensitive tumour and 7 out of 13 mice alive that had received the amethopterin-resistant *M*46*R* tumour. The animals that did die following re-inoculation with *L*1210 or the *M*46*R* variant showed a marked increase in survival-time as compared with the controls inoculated for the first time. In general, as had been observed earlier for leukæmia *L*1210 in immune mice[5], they succumbed with large local tumours at the site of inoculation. The control mice for both the sensitive and resistant sub-lines had median survival times of 12 days. (The control mice employed in this experiment were somewhat younger than the immune mice. However, as indicated earlier[5], the immune phenomenon could not be attributed to an age factor. In more recent studies, this has been re-checked for both leukæmia *L*1210*Z* and the *L*1210–*M*46*R* variant.) For both the sensitive and resistant sub-line of leukæmia *L*1210 the inoculum-level employed was well above that required to give 100 per cent takes in control mice inoculated with tumour for the first time. With the *L*1210*Z* the experimental inoculum was 10 times greater than that giving 100 per cent takes in controls, while with the *L*1210–*M*46*R* it was 100-fold greater than that required to give 100 per cent takes in controls.

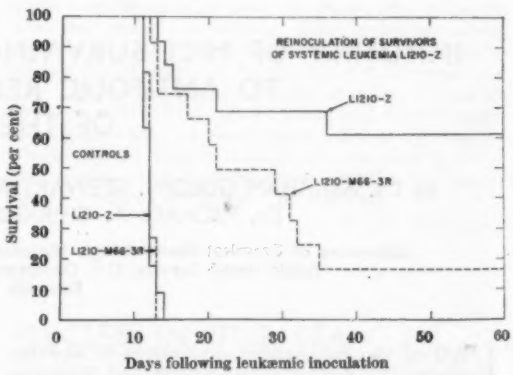

Fig. 2. Inoculation of the sensitive leukæmia (*L*1210*Z*) and a 3′5′-dichloroamethopterin-resistant subline (*L*1210–*M*66–3*R*) into *CDBA* mice. The re-inoculated mice had survived systemic leukæmia following treatment with 3′5′-dichloroamethopterin. Thirteen mice were re-inoculated with leukæmia *L*1210*Z* and 12 mice were re-inoculated with *L*1210–*M*66–3*R*. These mice were survivors of several experiments. All had been treated with 75 mgm./kgm. 3′5′-dichloroamethopterin starting at 7 days following leukæmic inoculation, and with one exception treatment was to the 50–80th day. Three of the mice re-inoculated with *L*1210 and four of the mice inoculated with *L*1210–*M*66–3*R* had already been shown to be immune to *L*1210. The remainder of the long-term survivors were re-inoculated for the first time. Controls, 11 mice/group of age comparable to that employed in the experiments. *L*1210*Z* and *M*66–3*R* inocula approximately 500,000 cells per mouse, subcutaneously. The inoculum concentration for both tumours was 100 times greater than that required to give 100 per cent takes in controls. *L*1210*Z* generation No. 267. *L*1210–*M*66–3*R* generation No. 20

Table 1. INOCULATION OF LEUKÆMIA *L1210Z* AND AN AMETHOPTERIN RESISTANT SUBLINE (*L1210–M46R*) INTO *BALB/c An* MICE IMMUNIZED AGAINST THE SENSITIVE LINE (*L1210Z*)

	First inoculation (*L1210Z*) Day 0			Second inoculation (*L1210Z*) Day 38			Third inoculation (*M46R*) Day 60		
	Takes / Total	Max. tumour diam. Av. mm.	Survivors / Total Day 38	Takes / Total	Max. tumour diam. Av. mm.	Survivors / Total Day 60	Takes / Total	Max. tumour diam. Av. mm.	Survivors / Total Day 128
BALB/c An	10/10	12·1	6/10	0/6	0	6/6	0/6	0	6/6
BALB/c An				10/10	10·5	9/10	0/9	0	9/9
BALB/c An							10/10	12	6/10
CDBA							12/12	10	0/12

Approximately 25 mgm. spleen inoculated per mouse (see ref. 5).

Fig. 2 summarizes results obtained when the variant *L1210–M66–3R* was utilized in a similar experiment. *CDBA* survivors of systemic leukæmia *L1210Z* exhibited immunity not only to subsequent inoculation of *L1012Z*, but also to the variant *L1210–M66–3R* resistant to 3′5′-dichloroamethopterin. Without any further therapy immune mice re-inoculated with each of these tumours showed extensive survival-time as compared with controls inoculated with either tumour for the first time.

It is of interest to note that the immune response appeared to be more extensive in mice re-inoculated with *L1210Z* as compared with *L1210–M66–3R*. There were 8 out of 13 sixty-day survivors on re-inoculation with *L1210Z* and 2 out of 12 sixty-day survivors following inoculation with *L1210–M66–3R*. By the same token the immunity to rechallenge with *L1210Z* appeared to be somewhat more extensive than to challenge with the *L1210–M46R* (Fig. 1). The nature of these differences is under further investigation.

Similarly, mice of strain *BALB/c An* immunized against leukæmia *L1210*, without drug therapy, were also immune to subsequent inoculation of the resistant variants *L1210–M46R* and *L1210–M66–3R*. This is illustrated for the *M46R* variant in Table 1. Group 1 had received the sensitive *L1210Z* as the first implant[4]. In 4 out of 10 mice the tumour grew progressively and the animals succumbed. In the remaining 6 mice the tumour regressed and the animals survived. On the second inoculation with leukæmia *L1210* the tumour failed to grow. The third inoculation was with *M46R* and the tumour did not take.

In group 2 (Table 1) the *L1210Z* had been inoculated once into the *BALB/c An* mice. The tumour grew locally and then regressed in all but one mouse. The nine survivors were inoculated with the *L1210–M46R* resistant variant and showed no evidence of tumour growth. In contrast, *BALB/c An* mice receiving the *M46R* variant for the first time (group 3) developed large local tumours at the site of inoculation, averaging 12 mm. in diameter. Four out of 10 mice succumbed to progressive tumour growth. In the remaining 6 animals the tumour regressed.

It is noteworthy that the *CDBA* mice and leukæmia *L1210* appear to be highly compatible. The immune response has not been observed in non-treated leukæmic *CDBA* mice. In this hybrid the leukæmia has never been observed to undergo spontaneous regression, and survivors of inoculum-levels too low to produce leukæmia have invariably been susceptible to re-inoculation.

In all the above experiments the source of inoculum was a suspension of leukæmic spleen taken from *DBA/2* mice, the strain of origin[8]. It is of interest to point out that attempts to immunize *CDBA* mice against *L1210Z* by pre-inoculation of *DBA/2* spleen from normal mice have been unsuccessful[5]. The immune response in *CDBA* mice has been observed only following successful therapy of leukæmia with the halogenated derivatives. Results were obtained suggesting that the immune response is not readily demonstrable unless successful therapy is initiated after the disease has become systemic[5].

On the other hand, transplantation of *DBA* spleen from normal mice into out-of-strain mice has been demonstrated to immunize mice against subsequent inoculation of *L1210* or the antifolic variants[7].

Since the leukæmic mice treated successfully with the dihalogenated derivatives of amethopterin developed immunity to the disease, it has been suggested that this immunity may contribute to the success of the drugs in chemotherapy[6]. Conceivably, chemotherapy may so alter or influence tumour cells that homograft characteristics are acquired or unmasked, thereby facilitating further therapy[5,9]. This would raise the question as to whether the origin of resistance to antifolic therapy is attributable to resistance to the drug *per se*, or to resistance to a therapeutically induced immune mechanism.

The current studies provide a separation between tumour cell resistance to therapy as opposed to tumour cell resistance to host immunity. The two variants of leukæmia *L1210*, *M46R*, and *M66–3R*, are resistant to antifolic therapy but are inhibited by the induced immunity of the host. Thus, although the immune mechanism may contribute to therapy, resistance to therapy does not necessarily imply abrogation of the effectiveness of the immune response. Interrelationships of chemotherapeutic and immunological phenomena in host-tumour systems are under further study. Elucidation of such phenomena may contribute to our knowledge of both transplantation and chemotherapy.

We wish to thank Gerome McDowell for capable technical assistance.

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REDUCTION OF INTESTINAL ABSORPTION BY A SYNTHETIC CHEMICAL

By Dr. J. A. NISSIM

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A SYNTHETIC bactericidal substance, trimethylhexadecylammonium stearate, was submitted to a study of pharmacological properties prior to a proposed trial in certain human alimentary infections. Preliminary studies showed loss of weight, which was first believed to be due to reduced food intake. Daily measurement of the food residue of control and treated groups showed no reduction in the food consumption of the latter, however, until a very late stage when the animals became moribund.

The animals used in these experiments were mainly adult male mice from non-interbred *C* stock. The water-insoluble trimethylhexadecylammonium stearate was incorporated in diet 41 (powder form), 5 gm. of which was given to each mouse per day. The concentration of the drug and the loss in weight after one month, or in the case of the three higher doses, at the time of death, are shown in Table 1. Analysis of variance gave $P \ll 0{\cdot}001$ for linear regression and for the variance between doses. Deviation from regression was not significant.

The histological appearances in mice receiving 0·4 and 0·5 per cent of the drug were compatible with the varying degrees of damage found in starved mice. In addition, most of the animals in these two groups showed at the time of death coffee-ground hæmorrhages in a specific area extending from the pyloric portion of the stomach to the ileum. This was invariably associated with damage to the intestinal villi, which involved primarily the epithelial lining. No hæmorrhages were noted in groups receiving smaller concentrations of the drug, though the animals still showed the appearances typical of inanition with total absence of depot fat and reduction in the weight of the testes and seminal vesicles ($P < 0{\cdot}001$). Animals receiving 0·1 per cent trimethylhexadecylammonium stearate died after 2–4 months of treatment, but some survived longer. The fæces were well formed in all groups, and the loss of weight could not, therefore, be attributed to intestinal hurry due to irritation. A few of the animals given 0·05 per cent of the drug for three months or more showed marked hypertrophy and hyperplasia of the duodenum and jejunum (Figs. 1 and 2).

Adult mice receiving 0·02 per cent trimethylhexadecylammonium stearate for six months did not differ from control mice either in weight or in general appearance. The rate of growth of immature mice given this concentration of the drug for periods of a fortnight at a time, in alternation with equal periods on diet 41, was depressed. The average fortnightly gain in weight per mouse was 2·5 gm. on the drug, as compared with 3·4 gm. for the control group ($P < 0{\cdot}001$).

Table 1. Weight Loss and Survival Period of Mice on Different Concentrations of Trimethylhexadecylammonium Stearate in the Diet

Conc. of drug in diet (gm. per cent)	No. and sex of mice	Original weight (gm.)	Weight at 1 month (gm.)	Difference (gm.)	Survival period	Average change in weight (gm.)
0·0						
a	6 m.	146·1	147·0	+0·9		
b	6 m.	140·4	140·5	+0·1		
c	6 m.	148·9	141·0	−7·9		
d	6 f.	128·6	125·7	−2·9		−0·41
0·025						
a	6 m.	139·3	141·7	+2·4		
b	6 m.	148·9	147·5	−1·4		
c	6 f.	128·1	127·9	−0·2		+0·04
0·05						
a	6 m.	145·8	144·0	−1·8	3 months or over	
b	6 m.	138·9	141·2	+2·3		
c	6 m.	149·6	150·0	+0·4		
d	6 m.	148·3	147·0	−1·3		
e	6 f.	128·4	125·0	−3·4		−0·12
0·1						
a	6 m.	146·0	138·0	−8·0	2–5 months	
b	6 m.	140·0	126·7	−13·3		
c	6 m.	148·5	138·0	−10·5		
d	6 f.	128·5	118·2	−10·3		−1·75
0·2						
a	6 m.	144·5	120·5	−24·0	17–35 days	
b	6 m.	140·2	114·5	−25·7		
c	6 m.	148·9	127·0	−22·9		
d	6 f.	126·9	117·8	−11·1		−3·49
0·4						
a	6 m.	153·0	118·0	−38·3	11–20 days	
b	6 f.	126·8	103·1	−23·7		−5·16
0·5						
a	6 m.	146·0	106·8	−39·2	3–12 days	
b	6 m.	134·4	110·7	−25·9		
c	6 m.	149·5	120·0	−29·6		
d	2 m.	54·5	39·5	−14·9		−5·48

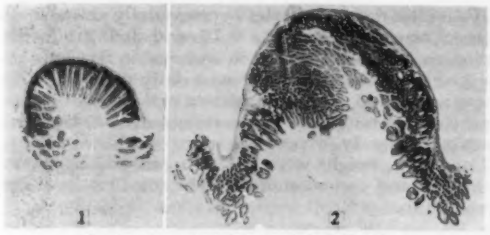


Fig. 1. Control : jejunum of normal mouse

Fig. 2. Jejunum of mouse kept on 0·05 per cent trimethylhexadecylammonium stearate in diet 41 for 3 months

A suspension of trimethylhexadecylammonium stearate in 0·5 per cent 'Tween 80' was administered to mice by stomach tube in doses of 25, 10, 5 and 2·5 mgm. daily. Given in this manner, the drug proved 3–5 times more effective than in diet 41. The animals died within 3, 7 and 9 days, and showed similar loss of weight and histopathological findings after the first three doses. Reduction of appetite became evident only in the terminal stages. With the latter dose, there was loss of weight comparable with that obtained on 0·1 per cent in the diet. The mice receiving this dose were alive and well after 10 weeks of treatment, though slimmer than a control group receiving 'Tween 80' only by the same route.

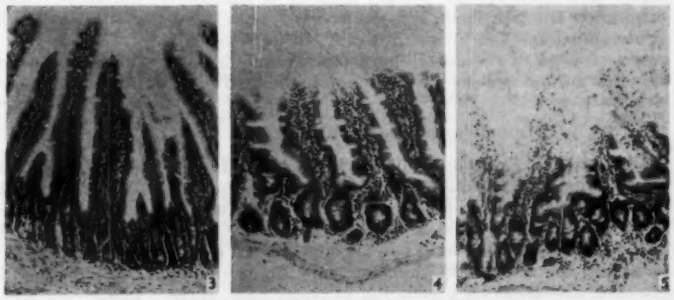
Trimethylhexadecylammonium stearate was more toxic when given subcutaneously in the form of the 'Tween' suspension. The *LD*50 was of the order of 0·6 gm./kgm. body-weight, a dose smaller than that received in a single day in a diet containing 0·5 per cent of the drug. After a dose of 1·0 gm./kgm., all the mice died within 24 hr., and 18/20 of these animals showed the characteristic intestinal hæmorrhages due to damage of the mucosa and villi in the pyloric area, duodenum and jejunum (Figs. 3–8). Single doses of 0·25 gm./kgm. did not cause death, but the animals continued to lose weight for some 10 days, and the difference in weight from a control group at one week was highly significant ($P<0·001$).

Administration via the intraperitoneal route proved more toxic still. The *LD*50 was of the order of 0·05 gm./kgm. for C_3H mice and 0·08 gm./kgm. for ordinary stock mice. Post-mortem usually showed marked dilatation of the stomach and intestine in addition to the previously described findings. Doses of 0·025–0·05 gm./kgm. given weekly for 10 weeks to eight mice on unrestricted diet resulted in gain of weight of 0·5 gm. as compared with 2·5 gm. per mouse in the control group.

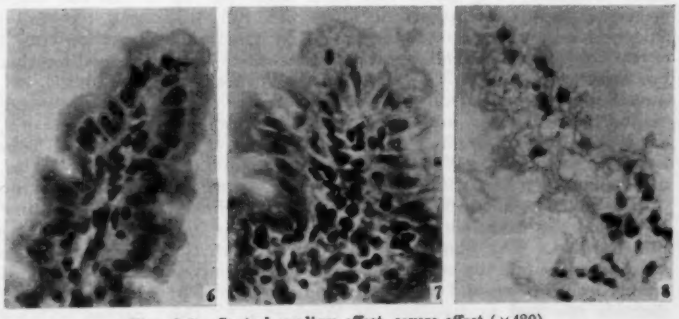
Animals allowed to recover after losing a fifth to a quarter of their body-weight (average loss = 4 gm.), either by continued administration of the drug in the diet (12 mice) or by a single subcutaneous administration (12 mice), were restored to normal weight when put on unrestricted normal diet. There was no obvious injurious effect on health up to three months after the end of the experiment, and the animals were indistinguishable from those in the control group.

The above results suggest that trimethylhexadecylammonium stearate has a specific effect on the mucosal cells of the villi in the pyloric, duodenal and jejunal regions of the gastro-intestinal tract, since the effect is obtained no matter what route is employed for administration of the drug. Large doses of this substance bring about the total destruction of mucosal epithelial cells and lead to the degeneration and collapse of the whole villus, whereas smaller doses appear to reduce the functional efficiency of the mucosal cell probably both in its absorbing as well as its digestive roles. The hypertrophy and hyperplasia following continued oral administration of 0·05 per cent of trimethylhexadecylammonium stearate in the diet seem to represent an effort on the part of the intestine to overcome the functional difficulty imposed by the drug. It would seem akin to the compensatory hypertrophy of a strained myocardium. The thickening is absent in the ileum and colon. This hypertrophy has also been noted occasionally after repeated intraperitoneal injection of the drug. (Intestinal hypertrophy was not produced by new batches of trimethylhexadecylammonium stearate, so that the exact composition of the batch and percentage of impurities may play an important part.)

A substance with such specific pharmacological activity opens up two important research fields. As a research tool it should prove a great aid in the study of intestinal absorption and the many enzymes



Figs. 3–5. Control, medium effect, severe effect (×60)



Figs. 6–8. Control, medium effect, severe effect (×420)

Figs. 4, 5, 7 and 8. Jejunums of mice showing moderate and severe damage to villi after subcutaneous injection of trimethylhexadecylammonium stearate (1·0 gm./kg m.)

that are probably concerned in this function. Furthermore, drugs which reduce food absorption may prove valuable in the control of obesity, atherosclerosis and coronary thrombosis.

Finally, the drug was tried on a healthy adult male volunteer. Three consecutive courses of the drug were administered at monthly intervals for periods of three, two and two weeks, respectively, and have resulted in loss of weight of 15, 9 and 7 lb., respectively. The drug was taken in the form of capsules in divided doses, and the average daily doses in the three courses were 1·0, 0·75 and 0·7 gm. This moderate dosage resulted in increase in the bulk and softness of the fæces, whereas a daily dose of 1·5 gm. was associated with mild nausea and fluid stools, but no colic or tenesmus. The effects of the drug were again evidently reversible, since, with over-eating, 7 and 4 lb. weight were put on rapidly at the end of the first and second courses. The total loss in weight was 20 lb.

α,ε-DIAMINOPIMELIC ACID: ITS DISTRIBUTION, SYNTHESIS AND METABOLISM

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THE introduction of paper partition chromatography by Consden *et al.*[1] made it possible to identify the components of many complex organic substances, and introduction of this technique stimulated numerous studies designed to characterize the amino-acid content of many natural products. One such study, first reported by Elizabeth Work[2], indicated the presence of a new ninhydrin positive material, designated as spot No. 17, in hydrolysates of both insoluble cell residues and, although in much lower concentration, in ethanol extracts of *Corynebacterium diphtheriae*. At about the same time an amino-acid with similar chromatographic behaviour was found in hydrolysates of soil[3], rumen contents of sheep (see footnote in Work[4]) and in a lipopolysaccharide fraction from *Mycobacterium tuberculosis*[5]. It remained for Dr. Work, however, in an excellent piece of work, to isolate, characterize and identify this new amino-acid as α,ε-diaminopimelic acid[6].

Properties and Distribution

Properties. Diaminopimelic acid is a neutral compound, moderately soluble in cold water and aqueous alcohol. It is soluble in dilute acid or alkali and is insoluble in ethanol and all organic solvents[4,6].

α,ε-Diaminopimelic acid can be synthesized chemically by the method of Sørenson and Anderson[7] or Sheehan and Bolhofer[8], yielding, in either case, three isomers, the DD, LL and *meso*. Separation of the three isomeric components has been achieved[9] by preparing the diaminopimelic acid diamide dihydrochloride, removing the *meso* diamide by fractional crystallization and resolving the DL racemate with hog kidney amidase[10]. The enzyme-treated racemate was then fractionated on the cation-exchange resin, 'Amberlite *XE*-64', and the respective isomers crystallized. The original procedure of Work *et al.*[6] has been modified by Wade *et al.*[11], who prepared the dicarbobenzoxy derivative which permits separation of the soluble dicarbobenzoxy-*meso*-diaminopimelic acid from the insoluble dicarbobenzoxy-DL-diaminopimelic acid. The diamide was prepared from the above compound, the carbobenzoxy groups removed by hydrogenation and the DL-diaminopimelic acid diamide resolved as described by Work *et al.*[9]. The dissociation constants of *meso* and LL-α,ε-diaminopimelic acid were identical: pk'_1 1·8, pk'_2 2·2, pk'_3 8·8, pk'_4 9·9, but differences in the infra-red spectrum of the two compounds in the region of 6–8μ were observed[11].

The distribution of the various stereoisomers of α-ε-diaminopimelic acid has been studied[12–14], and it is clear that all three isomers are found in microorganisms. The *meso* isomer or combinations of the *meso* and L-isomers seemed to be the most prevalent form of α,ε-diaminopimelic acid, although some of the *Propionibacteria*, *Streptomyces* and *Clostridia* contain only the L-isomer. The Micromonospora appear to contain all three isomers.

Distribution. Work[6] concluded that α,ε-diaminopimelic acid was probably restricted to protein of bacterial origin, since it had not been reported in the literature concerned with the chromatography of mammalian cell protein hydrolysates or tissue fluids. To check this hypothesis, Work and Dewey[15] conducted a survey of 118 micro-organisms, etc., including fungi, algae, protozoa and viruses, which revealed that α,ε-diaminopimelic acid was present in all the bacteria examined with the exception of the Gram-positive cocci. It was also found in two species of Myxobacteriales and in three species of Myxophyceae. It was absent, however, from yeast, fungi, two strains of protozoa, tobacco mosaic and turnip yellow mosaic virus. Knight[16], in a survey of the amino-acid composition of tomato bushy stunt, influenza *A* and *B*, Shope papilloma, cucumber virus and T_2 bacteriophage did not report the presence of α,ε-diaminopimelic acid in these viruses.

In the original survey[15], α,ε-diaminopimelic acid was reported to be absent from actinomyces and streptomyces but that these organisms contained instead another new amino-acid. This substance was designated as compound *A*. Work[17,18] presented evidence suggesting that compound *A* was a methyl homologue of α,ε-diaminopimelic acid, but subsequent studies[14], using the chromatography system of Rhuland *et al.*[19], revealed that compound *A* was LL-diaminopimelic acid. Cummins and Harris[20,21] and Cummins[22] reported that when α,ε-diaminopimelic acid was found in whole-cell hydrolysates, it was always present in the cell wall.

α,ε-Diaminopimelic acid has been found in soil hydrolysates[3], in caseous tuberculous nodules and caplan nodules[23], in pine tree pollen[24] and in alcohol extracts of neoplastic tissues[25]; but in all cases except the latter the presence of bacterial contamination could not be eliminated. Seibert *et al.*[25],

using an α,ε-diaminopimelic acid auxotroph, obtained growth of that organism in the absence of α,ε-diaminopimelic acid when drops of tissue hydrolysates were added to the bioautographic plates. These results must be re-examined with great care, however, since lanthionine was also present in the tissues, and this compound in the presence of trace amounts of α,ε-diaminopimelic acid, for example, when using an unwashed inoculum of *E. coli* 173–25, will support the growth of *E. coli* 173–25 (ref. 26). Seibert *et al.*[25] reported that lanthionine will support the growth of the *E. coli* auxotroph, but this was not confirmed by Rhuland[26] or Meadow *et al.*[27], using the same test organism.

With the finding of Salton[28] that α,ε-diaminopimelic acid exists in the cell walls of *E. coli*, *Salmonella pullorum* and *B. subtilis*, numerous reports have appeared revealing the presence of this amino-acid in various cell fragments. Smith *et al.*[29] found α,ε-diaminopimelic acid in the peptide fraction of a cell wall polysaccharide from *B. anthracis*, and its presence in a soluble peptide, produced by germinating spores of *B. megaterium*, was shown by Strange and Powell[30]. The recent review by Strange[31] should be consulted for a more detailed account of the occurrence of α,ε-diaminopimelic acid in various cell wall peptides of the bacilli. Koch and Dreyer[32] demonstrated the same amino-acid in fragments released by bacteriophage, as have Weidel and Primosigh[33]. A recent publication by Strominger[34] indicated that *meso*-α, ε-diaminopimelic acid occurs in a 'Park's type nucleotide' (Park[35]) in *E. coli*.

With the exception of the demonstration of α,ε-diaminopimelic acid in *Corella ellipsoidia* by Fujiwara and Akabori[36], it appears that the distribution of this amino-acid is limited to the Schizomycetes and the Myxophophyceae, thus lending support to the thesis of Stanier and VanNiel[37] that these groups should be combined into one kingdom, the Monera.

Isolation. The lysine auxotroph, *E. coli* 26–26, isolated by Davis[38], accumulates large amounts of α,ε-diaminopimelic acid in the culture medium and can be used as a source of *meso* and LL-α,ε-diaminopimelic acid. Wright and Cresson[39] used an ion-exchange chromatography procedure involving adsorption of the amino-acid on 'Dowex-1' (OH^-) followed by elution with 0·2 *N* hydrochloric acid, removal of anions in the α,ε-diaminopimelic acid fractions with 'Dowex-1' (HCO_3^-) and finally deionization with 1*R*-4*B* (OH^-) and 1*RC*-50 (H^+). Crystallization of α,ε-diaminopimelic acid was achieved by adding ethanol to concentrated solutions of α,ε-diaminopimelic acid after final de-ionization.

Work and Denman[40] used a somewhat simpler procedure employing acid-washed 'Zeokarb-225'. Contaminating amino-acids, aspartate, glutamate, glycine, alanine and valine were removed first with 1·5 *N* hydrochloric acid. Some 2·5 *N* hydrochloric acid was then added to elute the α,ε-diaminopimelic acid. The amino-acid hydrochloride was crystallized directly from the concentrate or obtained as the free base after removal of chloride ion with deacidite. I have used this same procedure, except that 'Dowex-50' (200–400 mesh) was used as the resin, to obtain α,ε-diaminopimelic acid labelled with carbon-14 from culture filtrates of *E. coli* 26–26 grown on uniformly labelled glucose. Ikawa and O'Barr[41] used the 'Dowex-50' procedure to obtain *meso*-diaminopimelic acid from acid hydrolysates of *Lactobacillus arabinosus*.

Since *E. coli* produces *meso* and LL-α,ε-diaminopimelic acid, both isomers are obtained by these resin procedures, although, as shown by Wright and Cresson[39], the LL isomer may be removed by recrystallization. Hoare and Work[19] attempted to separate the two isomers by the procedure of Work and Denman[40], but were unable to achieve a practical method of separation.

Figueroa and Seibert[42] studied the chromatography of α,ε-diaminopimelic acid on starch columns, and found that this procedure does not separate α,ε-diaminopimelic acid from lysine.

E. coli 26–26 and high-producing mutants derived from it have been used in the commercial production of lysine[43]. In this patented process toluene-treated cells of *A. aerogenes* are added to fermentation broths containing large quantities of α,ε-diaminopimelic acid; the lysine, obtained after decarboxylation of the α,ε-diaminopimelic acid, is isolated by resin chromatography.

Metabolism

Oxidation. Natural diaminopimelic acid from *Corynebacterium diphtheriae*, the *meso* isomer, was shown by Burton[44] to be oxidized by the L-amino-acid oxidase of *Neurospora crassa* with the consumption of one atom of oxygen and the liberation of one molecule of ammonia per molecule of amino-acid (see also Work[6]). Bonetti (personal communication to Work[6]) reported that this same preparation was not oxidized by kidney D-amino-acid oxidase or ophio L-amino-acid oxidase. Subsequent studies by Work[45] revealed that the *Neurospora* oxidase attacks *meso*, LL-α,ε-diaminopimelic acid and the L-diaminopimelic acid-D-monoamide at about the same rate. The L-amino-acid oxidases of viper and rattlesnake attacks the *meso* and L-isomers at a much slower rate than the *Neurospora* oxidase, but metabolized the L-diaminopimelic acid-D-monoamide at a rate almost comparable with the *Neurospora* preparation. The DD-isomer was not attacked nor did it have any effect on the course of oxidation of the other isomers.

Racemization. A racemase, present in acetone-dried cells of *E. coli* 26–26 (lysine auxotroph), converts L-diaminopimelic acid to the *meso* isomer[46]. Subsequent studies[47,48] have revealed some of the properties as well as the distribution of this enzyme. Diaminopimelic acid racemase acts on either *meso* or L-diaminopimelic acid, but has no activity on the DD-isomer. As Antia *et al.*[48] pointed out, the substrate for this enzyme is unusual in that it has two asymmetric carbon atoms which are symmetrical about the γ-carbon atom. The experimental evidence presented indicates that one end of the α,ε-diaminopimelic acid molecule must be in the L-configuration to ensure enzymatic activity.

Diaminopimelic acid racemase was found in almost all bacteria containing α,ε-diaminopimelic acid, although there was no correlation between the type of isomer and racemase content[48]. It is noticeably absent from certain *Clostridia*, but present in several of the Micrococci which do not contain α,ε-diaminopimelic acid.

Decarboxylation. The structural relationship of α,ε-diaminopimelic acid and lysine and the established presence of amino-acid decarboxylases in bacteria[49] suggested an important metabolic relationship between these two amino-acids involving a possible mechanism of lysine biosynthesis in bacteria.

Dewey and Work[50] demonstrated that α,ε-diaminopimelic acid was indeed decarboxylated to lysine by some strains of *E. coli*. The enzyme, as most decarboxylases, was most active in cells grown at acid *p*H, but unlike the previously reported cases, was constitutive rather than adaptive. Davis[35] implicated α,ε-diaminopimelic acid as a precursor of lysine with his finding that a number of lysine auxotrophs accumulated large amounts of α,ε-diaminopimelic acid in the culture supernatant. Vogel[51], studying the metabolism of [4-^{14}C] aspartate in strains of *Hydrogenomonas facilis*, *Pseudomonas fluorescens*, *Azotobacter agilis*, *Agrobacterium radiobacter*, *Alcaligenes faecalis*, *Escherichia coli*, *Micrococcus lysodeikticus*, *Arthrobacter globiformis*, *Bacillus subtilis* and *Streptomyces griseus*, found what appear to be a single over-all mode of lysine biosynthesis, presumably via α,ε-diaminopimelic acid. Similar studies with *Chorella vulgaris* and *Euglena gracilis*[52] revealed that the *Chorella* containing α,ε-diaminopimelic acid synthesize lysine via the α,ε-diaminopimelic acid pathway, while the *Euglena* not containing α,ε-diaminopimelic acid utilized the fungi pathway of lysine biosynthesis, thus lending support to the thesis advanced by Dewey and Work[50] and Davis[35]. Recent experiments by Meadow and Work[53], using an auxotroph of *E. coli* that requires only α,ε-diaminopimelic acid for growth, report that, in this organism, lysine can be synthesized by a metabolic sequence that does not proceed via α,ε-diaminopimelic acid. This conclusion is based on the finding that this auxotroph, *E. coli* 173-25-*D*, grown on glucose labelled with carbon-14, incorporated radioactivity in the cellular lysine, but not the α,ε-diaminopimelic acid.

With the implication of α,ε-diaminopimelic acid as an intermediate in the biosynthesis of lysine it was important not only to determine the distribution of this enzyme, but also to ascertain with certainty that the decarboxylation of α,ε-diaminopimelic acid was not due to a non-specific lysine decarboxylase. That a specific α,ε-diaminopimelic acid decarboxylase was present was clearly demonstrated by Dewey and Work[50] using the lysine auxotroph isolated by Davis[35] which contained lysine decarboxylase, but no α,ε-diaminopimelic acid decarboxylase. Additional proof was obtained by growing *E. coli* in a glucose salts medium which, under these conditions, contains no lysine decarboxylase but does contain the constitutive α,ε-diaminopimelic acid decarboxylase.

Dewey and Work[54] reported the presence of α,ε-diaminopimelic acid decarboxylase in *Aerobacter aerogenes* and, in a later paper, Dewey[55] surveyed a number of strains of the *coli-aerogenes* group and other bacteria for the presence of this enzyme. The results of the survey indicated that the enzyme is widely distributed in the *coli-aerogenes* group. The enzyme was also found in a strain of *Pseudomonas aeruginosa*, but not *Proteus vulgaris*, *Streptococcus faecalis* or *B. subtilis*. Work[56] and Dewey *et al.*[57], in further studies of this type, reported the presence of the enzyme in *Actinomyces* sp., *Rhodopseudomonas spheroides*, *Rhodospirillum rubrum* and in three organisms which do not contain α,ε-diaminopimelic acid, *Sarcina lutea*, *Staphylococcus aureus* and *Streptococcus faecalis*. Powell[58] has reported the enzyme to be present in spores of *B. sphaericus*, as has Willett[59] in *Mycobacterium tuberculosis*.

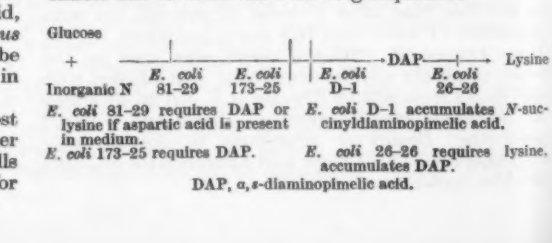
Diaminopimelic acid decarboxylase, unlike most amino-acid decarboxylases, is a constitutive rather than adaptive enzyme[50] and, although formed in cells grown at an acid *p*H, must be assayed at *p*H 6·8 for maximum activity[60]. The enzyme is specific for α,ε-diaminopimelic acid and will not decarboxylate a number of closely relative compounds such as diaminoadipic acid, diaminosuberic acid, α,ε-diaminoβ-hydroxypimelic acid, lanthionine or pyridine-2,6-dicarboxylic acid[57,60].

Diaminopimelic acid decarboxylase can be partially purified by fractional extraction with acetone[60] and, although inhibitor studies have shown that it is a very sensitive sulphydryl enzyme, the co-factor requirements have not been ascertained. Pyridoxal phosphate is required for maximal activity[60], but an absolute requirement for this coenzyme has not been demonstrated. The α,ε-diaminopimelic acid and lysine decarboxylase levels in pyridoxin-deficient *E. coli* have been determined[61], but the results failed to show the expected absolute requirement for pyxidoxal phosphate. Similar results were obtained by Powell and Strange[62] with *B. sphaericus*. Meadow and Work[63] studied the α,ε-diaminopimelic acid decarboxylase from *B. sphaericus* in more detail, using a number of pyridoxine inhibitors, but again were unable to demonstrate the absolute requirement of pyridoxal phosphate in this reaction. These workers concluded that there was a low affinity between enzyme and pyridoxal phosphate in *B. sphaericus*.

Initial studies with α,ε-diaminopimelic acid decarboxylase revealed that the enzyme was relatively specific for *meso*-α,ε-diaminopimelic acid, but that acetone-dried cells of *A. aerogenes* decarboxylated LL-α,ε-diaminopimelic acid[13]. Activity against the L-isomer was not always obtained, and Hoare[44] resolved this difficulty with the finding that the apparent decarboxylation of LL-α,ε-diaminopimelic acid was due to its conversion to the *meso* isomer by racemase activity. The D-isomer was not metabolized by α,ε-diaminopimelic acid decarboxylase[46,48].

Transamination. With the widespread distribution of transaminases in bacteria, it is perhaps not unreasonable to expect that α,ε-diaminopimelic acid could act as an amino donor in such a reaction. Evidence demonstrating this reaction was reported by Meadow and Work[64] using acetone-dried cells of *E. coli*, *B. cereus*, *B. sphaericus*, *S. lutea*, *A. aerogenes* and *S. saprophyticus*. Contrary to the racemase, oxidase and decarboxylase reactions, the D-isomer was shown to participate in the transaminase reaction and in the *S. lutea* preparation was transaminated faster than the *meso* isomer. In general, pyruvate, oxalacetate and oxoglutarate act as amino acceptors, but the rate varies with the organism and with the stereoisomer used. Using *B. sphaericus*, Meadow and Work[64] reported that concentrations of pyridoxal phosphate from 1μ to 100μ moles progressively increased the rate of transamination.

Biosynthesis. It is perhaps pertinent to start a discussion of α,ε-diaminopimelic acid biosynthesis with a description of the bacterial mutants which have been used to elucidate in part the mechanism of α,ε-diaminopimelic acid biosynthesis. A number of these mutants have been reported by Davis[35] and enable one to write the following sequence:

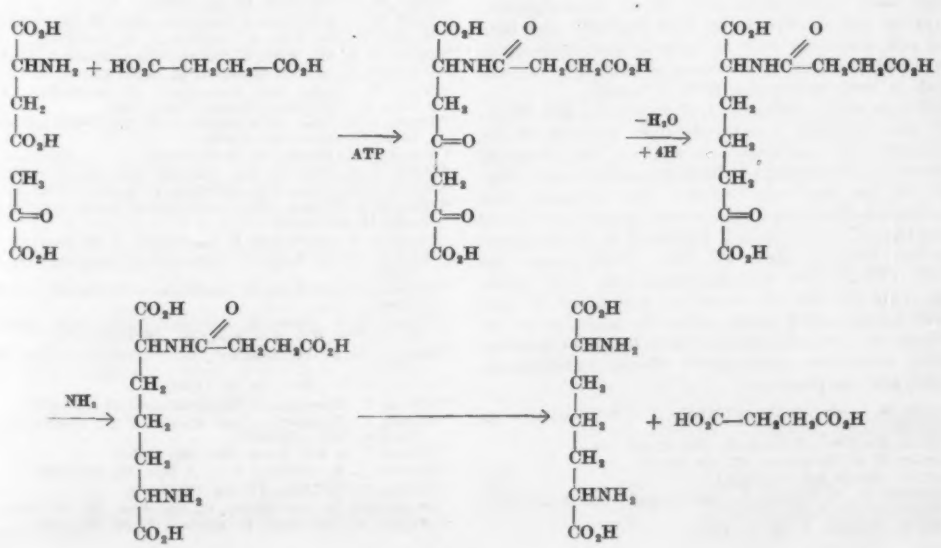


As a result of studies of amino-acid biosynthesis with various compounds containing carbon-14, Abelson *et al.*[65] were able to demonstrate that four of the carbon atoms of α,ε-diaminopimelic acid and lysine were derived from aspartic acid. In these studies aliquots of cell hydrolysates were subjected to paper chromatography and the specific activity of the radioactive amino-acids determined directly on the paper. Rhuland and Bannister[66], using *E. coli* 26-26 (accumulates α,ε-diaminopimelic acid) culture filtrates, isolated a compound capable of supporting the growth of *E. coli* 81-29 in the absence of α,ε-diaminopimelic acid. The compound was identified as succinic acid and, as shown by the same authors, was required for maximal synthesis of α,ε-diaminopimelic acid by cell-free preparations of *E. coli* 26-26. In addition to succinic acid, aspartate, pyruvate, adenosine triphosphate and triphosphopyridine nucleotide were also shown to be required in this synthesis. Similar results were reported by Gilvarg[67] with the exception that both di- and tri-phosphopyridine nucleotide were indicated as stimulating α,ε-diaminopimelic acid synthesis, and glutamate instead of succinate was listed as one of the components of the reaction. Subsequent studies by Gilvarg[68] reported the isolation of *N*-succinyl-L-diaminopimelic acid from *E. coli D*-1. This compound was enzymatically hydrolysed to succinic acid and α,ε-diaminopimelic acid by extracts of *E. coli* 26-26 and *E. coli W* (wild type), but not *E. coli D*-1, thus indicating its role as an intermediate in the synthesis of α,ε-diaminopimelic acid and offering an explanation for the function of succinic acid as a growth factor for an α,ε-diaminopimelic acid auxotroph[66]. Additional studies[69] have revealed that the succinyl side-chain is attached to the aspartate portion of N-succinyl-L-diaminopimelic acid.

Gilvarg[70], in a more detailed report of his original observation, confirmed the results of Abelson *et al.*[65], demonstrating that in cell-free extracts, as well as in growing cells, aspartate contributes four of the carbon atoms in the α,ε-diaminopimelic acid molecule. In this study, contrary to the previous report[67], triphosphopyridine nucleotide was indicated as the coenzyme of choice, thus confirming the results of Rhuland and Bannister[66] and Rhuland and Soda[71]. Although the original preliminary report of Gilvarg[67] did not point out which coenzyme was most effective, the coenzyme requirement may depend on the condition of the cells used for preparing α,ε-diaminopimelic acid synthesizing enzymes since Rhuland and Soda[71] showed that diphosphopyridine nucleotide is the preferred coenzyme in extracts from aged cells, but not in extracts from fresh cells. It does not seem likely, however, in view of the results of Rhuland and Soda[71] indicating that glutamate is no more active, with the exception of succinate, than the other Krebs cycle compounds in stimulating α,ε-diaminopimelic acid synthesis, that this compound is used *per se* in this synthesis. It would seem that this evidence and a consideration of the facts that acetyl or formyl aspartate are not used in the reaction[70] along with the isolation of *N*-succinyldiaminopimelic acid are adequate indications of the role of succinic acid in the reaction sequence under discussion.

The accumulation of 2,N-succinyl-6-ketopimelic acid, in addition to N-succinyldiaminopimelic acid, by *E. coli D*-1 is described by Gilvarg[72]. This compound can be transaminated to N-succinyl-L-diaminopimelic acid by extracts of *E. coli* using glutamic acid as the amino donor. Another brief report[72] described the purification of the transaminase and its specificity for glutamic acid as the amino donor. This transaminase has also been detected in *A. aerogenes*, *A. agile*, *M. lysodeikticus* and *R. spheroides*.

Rhuland and Soda[71] presented evidence revealing that an N-substituted-keto-pimelic acid was made by extracts capable of synthesizing α,ε-diaminopimelic acid. The compound was converted to α,ε-diaminopimelic acid after reductive hydrogenolysis of the 2,4-dinitrophenylhydrazone followed by acid hydrolysis. These results have enabled the above authors to write a tentative mechanism for α,ε-diaminopimelic acid biosynthesis which up to the present time appears to be compatible with the experimental results reported in the literature. The mechanism is as follows.



It is clear from a consideration of this mechanism that the synthesis of α,ε-diaminopimelic acid from small carbon fragments involves seven or eight enzymatic steps with the formation of some new and novel organic compounds. However, it is interesting to note that the enzymatic studies reported thus far have not indicated co-factor requirements other than triphosphopyridine nucleotide. I have achieved a ten-fold purification, involving dialysis, protamine and ammonium sulphate fractionation, of the α,ε-diaminopimelic acid synthesizing complex without demonstrating the need for additional co-factors. The abstract of Peterkofsky and Gilvarg[73] indicated a 150-fold purification of the N-succinyl-ε-ketopimelic-glutamic transaminase; but did not mention a pyridoxal phosphate requirement for this system.

Experiments similar to those described by Abelson *et al.*[65] have been interpreted by Meadow and Work[74] to indicate two pathways of α,ε-diaminopimelic acid biosynthesis. These conclusions were based on the fact that *E. coli* 26-26 grown with unlabelled glucose and aspartate labelled with carbon-14 and/or pyruvate incorporated radioactivity into cellular α,ε-diaminopimelic acid but not in the exogenous α,ε-diaminopimelic acid. Use of trace amounts of labelled acetate under the same conditions, however, resulted in radioactivity in both the cellular and extracellular α,ε-diaminopimelic acid. These observations and the fact that extracellular α,ε-diaminopimelic acid was not produced until the period of maximum growth was attained enabled the above authors to conclude that cellular α,ε-diaminopimelic acid was synthesized by one metabolic pathway and that extracellular α,ε-diaminopimelic acid was synthesized from different precursors.

These studies do not negative any of the findings on α,ε-diaminopimelic acid biosynthesis described above; but it would appear that further investigations will be necessary to establish with certainty that two different synthetic pathways for α,ε-diaminopimelic acid are operative.

Elucidation of the steps involving activation of succinate, aspartate and pyruvate and the identification of all the components in this metabolic sequence, as well as the many aspects of α,ε-diaminopimelic acid metabolism still to be investigated; activation and incorporation into peptides and bacterial cell walls, its role in lipopolysaccharide complexes and the specific function of each isomer, will provide a very interesting field of study.

Added in proof. Gilvarg (*J. Biol. Chem.*, **234**, 2955; 1959) has reported a more detailed account of the production and isolation, as well as the chemical synthesis, of N-succinyl-L-diaminopimelic acid. Contrary to his previous report[65], he indicates that N-succinyl-L-diaminopimelic acid is actually produced by mutant *E. coli* D-1$_{(a)}$. Rhuland and Hamilton (*Biochim. Biophys. Acta*, **36**, 559; 1959) report that *E. coli* 173-25, the α,ε-diaminopimelic acid auxotroph, contains all the enzymes necessary to synthesize α,ε-diaminopimelic acid. In addition to the synthesis of LL-diaminopimelic acid by this organism, another substance, presumably N-succinyl-diaminopimelic acid, is produced.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Low-Energy X-Rays from Interplanetary Space

It is generally believed that the Sun is a source of highly ionized gas which streams outward through the solar system at all times and in all directions[1]. At the orbit of the Earth, even under the quietest solar conditions, the velocity and density appear to be of the order of 500 km./sec. and 10^2 to 10^3 particles/cm.^{3} respectively. (When the Sun is very active velocities greater than 1,500 km./sec. and densities up to 10^5 cm.^{3} may be produced sporadically.) Theoretically[2], it is expected that this steady gas stream will finally become unstable at some radial distance from the Sun and establish a region of highly disordered interplanetary magnetic field and plasma.

I wish to point out that energy interchange processes between the heavy ions and the electrons of the plasma which may be expected to occur in this disordered region of hydromagnetic turbulence should lead to a diffuse source of low-energy cosmic X-rays which may be observable at altitudes greater than about 120 km. Exchange processes available, in addition to the Fermi and betatron acceleration mechanisms, include excitation of plasma oscillations[3] and a general process described by Veksler[4] which may operate as a plasma encounters any inhomogeneous magnetic field. The Veksler mechanism can, in principle, lead to complete interchange of energy between the protons and the electrons of the streaming solar plasma, producing thereby electrons with energies somewhat in excess of 1 keV. The total energy W_γ in the form of bremsstrahlung photons radiated by these electrons per c.c. of the disordered region, if only $Z = 1$ ions are considered, is given by :

$$W_\gamma = 11{\cdot}9 \times 10^{-30} N^2 \overline{T}^{\frac{1}{2}} \text{ ergs/cm.}^3\text{/sec.}$$

Presence of He and higher Z ions in the interplanetary gas will be neglected although such ions can probably contribute appreciably to the radiated power. $\overline{T}$ is the average energy of the electrons in ergs.

I now consider only photons between T and αT, where α is an instrumental cut-off, taking the electrons to be mono-energetic with energy T. The total number of such photons arriving near the Earth may be computed if we assume spherical symmetry, a disordered region extending radially[3] from 2 to 5 A.U., and complete energy exchange (without the inverse process) over a distance short compared to the thickness of the disordered region. Absorption is negligible since the total Thompson scattering probability in a path 1 A.U. in length is only $\sim 10^{-7}$. Under these conditions, the X-ray number flux F_γ near the orbit of Earth is given by :

$$F_\gamma = \frac{11{\cdot}9 \times 10^{-30} \log_e\left(\frac{1}{\alpha}\right)}{T^{\frac{1}{2}}} a^4 \int_{2a}^{5a} N_0^2 \, r^{-4} \, dr$$

where a is the Sun–Earth distance $= 1{\cdot}5 \times 10^{13}$ cm. $= 1$ A.U. and N_0 is the plasma particle density near the Earth.

If we consider X-rays between 10 and 50 Å., which may be readily measured with rocket or satellite-borne proportional counters, then $\alpha \approx 0{\cdot}2$ and with $N_0 = 10^3$ we find $F_\gamma \simeq 23$ X-rays/cm.^{2}/sec. corresponding to an energy flux of $\sim 3 \times 10^{-8}$ ergs/cm.^{2}/sec. Note that if N_0 is closer to 10^2 than 10^3 the flux will be $\sim 3 \times 10^{-6}$ ergs/cm.^{2}/sec. or $2{\cdot}3 \times 10^3$ photons/cm.^{2}/sec. (Radiation which may originate from individual streams of exceptional velocity and density such as are produced during periods of great solar activity is discussed elsewhere. Reiffel, L. (to be published).) These are, of course, upper limits to the flux, on the assumption that complete energy transfer occurs, which is obviously improbable. However, on the night side of the Earth, provided background radiation from celestial sources is not serious, rather high sensitivity might be achieved, hence the radiation may be detectable even in the event of a low energy interchange efficiency. Solar background radiation, backscattered into the detectors, would be low because of the small total scattering probability per A.U. mentioned earlier. Collimated detectors might be used to advantage to look for structure, for example, higher intensity in the plane of the ecliptic or 'patchiness' in the interplanetary gas X-ray source, and to discriminate against stellar backgrounds and auroral X-rays from the Earth.

To my knowledge, the only experimental information on X-rays in the night sky is the negative result obtained in a measurement briefly mentioned by Friedman[5] and obtained in the course of the N.R.L. rocket programme. The upper limit, established by the lack of a measurable flux, in this case was 10^{-6} ergs/cm.^{2}/sec. per Å. in the region around 50 Å. The counters used for the measurement were presumably of the type described by Byram *et al.*[6] and, as such, were more appropriate to detecting the high fluxes associated with direct exposure to solar X-rays. The upper limit quoted above corresponds to an energy flux, calculated on the basis of a thin target bremsstrahlung spectrum, of about 2×10^{-6} ergs/cm.^{2}/sec. over the wave-length range 10–50 Å. This is just about the upper limit calculated for the X-rays of interplanetary origin on the assumption of the higher gas density. Presumably counters specifically designed for this task could be made a great deal more sensitive since the effective area of the counters used by Byram *et al.* was only of the order of 0·05 cm.^{2} while the efficiency was about 5 per cent.

The possibility of observing radio noise from the mechanism described above will be discussed in a subsequent communication.

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Armour Research Foundation,
Chicago, Illinois. Dec. 16.

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GEOPHYSICS

Observations on the Solar Eclipse of October 2

DURING the recent solar eclipse of October 2, 1959, measurements were made at the U.S. Air Force Cambridge Research Center's radio astronomy site at Hamilton, Massachusetts. At this location the Sun rose partially eclipsed and 10 min. later it was totally eclipsed at an elevation of about 1°.

Having tracked the Sun from sunrise for a week prior to the eclipse, average refraction corrections were worked out to permit accurate antenna pointing and tracking of the Sun.

Measurements were made simultaneously at 224 and 1,300 Mc./s., using an 84-ft. paraboloid, and at 3,000 Mc./s. using an 8-ft. dish located 80 ft. east of the 84-ft. antenna.

Information was obtained from the records of the eclipse and in comparison with records of days both before and after the eclipse (control days).

On the eclipse day, the Sun was quiet. In fact, it was at its coldest level for the two weeks observed. However, interferometric solar maps at 3,000 Mc./s. (R. Bracewell, personal communication), at 9,300 Mc./s. (J. F. Denise, personal communication) and at 1,420 Mc./s. (W. N. Christiansen, personal communication) indicate the presence of plage areas on both the east and west limbs of the Sun. From this, one would expect the apparent solar diameter to be larger than the quiet Sun in the shorter wave-length region, with high-intensity levels extending beyond the solar disk.

This accounts for the fact that the residual energy recorded at totality at both 3,000 Mc./s. and 1,300 Mc./s. of 20 per cent of the uneclipsed Sun is greater than the signals recorded during low sun-spot cycle year eclipses. At 224 Mc./s., however, with radiation originating in the corona little affected by the plage areas, and with interferometric maps

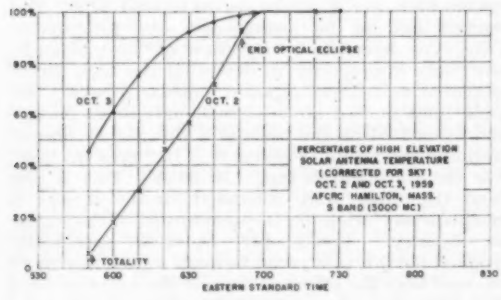
END OPTICAL ECLIPSE
OCT. 3
OCT. 2
TOTALITY
PERCENTAGE OF HIGH ELEVATION SOLAR ANTENNA TEMPERATURE (CORRECTED FOR SKY) OCT. 2 AND OCT. 3, 1959 AFCRC HAMILTON, MASS. S BAND (3000 MC)
EASTERN STANDARD TIME

Fig. 1

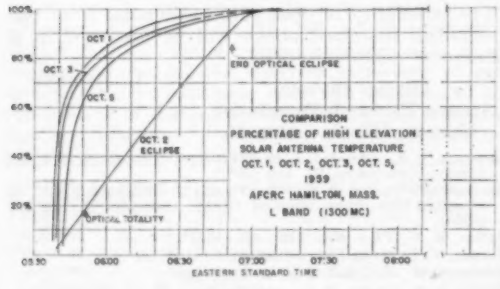
OCT. 1
OCT. 3
OCT. 5
END OPTICAL ECLIPSE
OCT. 2 ECLIPSE
COMPARISON PERCENTAGE OF HIGH ELEVATION SOLAR ANTENNA TEMPERATURE OCT. 1, OCT. 2, OCT. 3, OCT. 5, 1959 AFCRC HAMILTON, MASS. L BAND (1300 MC)
OPTICAL TOTALITY
EASTERN STANDARD TIME

Fig. 2

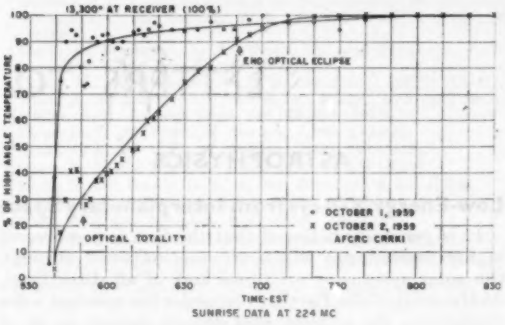

Fig. 3

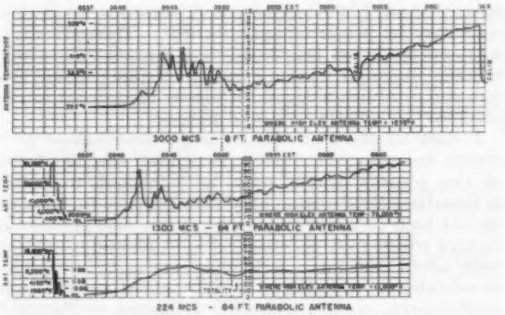

Fig. 4

plotted at this frequency-range at Nançay, France, for example, indicating no point source of solar activity, the residual energy-level of about 35 per cent at totality was not unlike the value for low sun-spot cycle year eclipse records.

Energy-levels during the recent eclipse are plotted in Figs. 1, 2 and 3.

Fig. 4 is a record of the signals received in the early part of the eclipse period. Since totality occurred when the Sun's elevation was approximately one degree, low-angle effects were evident. Large scintillations were seen starting at about 0542 E.S.T., especially at the higher frequencies. At this time, the Sun was rising with the east limb as yet uneclipsed. Two radio-frequency active plage areas in this region account for the high-amplitude scintillations. Normally with an uneclipsed solar disk the active plage areas act as point sources and at elevations up to 4 or 5° produce fairly deep fluctuations as, indeed, they did on all days observed. However, none could approach the violence of the 1,300 Mc./s. fluctuation at 0542 E.S.T., Oct. 2, 1959, in terms of percentage of antenna temperature, which was 5 or 6 times the mean curve temperature. Throughout the entire period illustrated, scintillations were high due to early covering and later uncovering of point sources on the Sun with scintillations existing even during 'totality'.

Individual fluctuations at 1,300 Mc./s. and 3,000 Mc./s. were remarkably well correlated in detail, in spite of the separation of the two antennæ and the difference in size of antenna apertures. Thus the peaks and nulls of one frequency are well matched by the other throughout the entire control period.

During the control period, no scintillations were recorded at 224 Mc./s. This is consistent with th

quiet nature of the Sun and its large angular diameter at this frequency.

From the scintillation records, two points are evident. The first—the shadow patterns at 1,300 Mc./s. and 3,000 Mc./s. (generated by the radio-frequency active areas on the Sun, which interfering with one another normally decrease the amplitude of the scintillations) are not dependent on frequency. This point is somewhat at variance with work reported by Kazès[1]. The second—within the distance between the centres of the two antennæ, a single shadow pattern exists. This latter observation is generally in accord with work of Steinberg and Kazès[2].

A more detailed analysis of the eclipse and scintillation data will appear at a later date.

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Rare Hiss, Earth Currents and Micropulsations on November 27, 1959

On November 27, 1959, a rare signal was heard on the chorus-whistler equipment[1] at College, Alaska. Beginning as a whistle of perhaps 2 kc./s. at approximately 1351 A.S.T. (2351 G.M.T.) on November 27, 1959, a tone rose slowly to about 10 kc./s., becoming a hiss lasting for what seemed to be several minutes. At the same time magnetic field micropulsations[2] with periods of 10–20 sec. were initiated with a commencement magnitude of about 2·5γ falling to 0·4γ in about 5 min.

An unusually abrupt sudden commencement on the Earth current record[3] at College was also observed at 1351 A.S.T. Although sudden commencements are not rare in the Earth current records, they are usually preceded by some sort of activity. During the hour before the sudden commencement the maximum range of activity in the geographical north–south component was 16 mV./km. At 1351 the trace was abruptly displaced—572 mV./km. In less than 30 sec. the trace recovered and went + 410 mV./km. A negative deflexion of the trace corresponds to a northerly directed electron current in the Earth. For the next 3 hr. the trace oscillated about 150 mV./km. with a dominant period of about 2 min. Strong activity continued through the night, and storms occurred during the next two nights.

A red auroral arc was observed later that day commencing in the north about 1645 A.S.T., reaching the zenith at about 1720 and fading by 1745.

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Continental Drift

In recent years the question of continental drift has received renewed attention mainly as a result of palæomagnetic studies. The general consensus of opinion appears now to look with favour upon this hypothesis. No attempt will be made here to summarize all the supporting evidence—rather the purpose of this communication is to point out a difficulty with such a theory. This is based in part on measurements of the flow of heat through the Earth. Certainly far too few heat-flow measurements have been made to draw any quantitative conclusions. Nevertheless, in spite of quite a large range in the measurements of the flow of heat through the ocean floors, the average flow from oceanic and continental areas is approximately the same. There are also many cases where the oceanic flow of heat is greater than the average value through the continents. These results were unexpected on the basis of the radioactive content of typical oceanic and continental crustal rocks existing near the Earth's surface.

It is a very real problem to find sources of heat beneath the ocean floors. The whole of the observed continental heat flow could be produced by the uranium, thorium and potassium contained in about 25 km. of granite. Granitic type rocks are almost completely absent under the oceans—typical oceanic rocks being basalt the radioactive content of which is only about 30 per cent of that of continental rocks. Bullard, Maxwell and Revelle[1] have shown that heat production in the sediments due to biological activity, radioactivity and other processes such as chemical weathering cannot account for more than 1 per cent of the observed values. Again, the oceanic crust, being relatively thin and composed mainly of basaltic materials, can produce at most 10 per cent of the observed values. The remaining heat must therefore come from the mantle. There is thus a striking difference between the mantle under the oceans and under the continents. Hence, if a continent, with its more radioactive crust, were to drift over an oceanic area, with its more radioactive mantle, much higher continental heat flows than are observed would be expected.

The distribution of heat sources in the mantle under the oceans is quite critical if melting at depth is to be avoided. A distribution of radioactivity to depths greater than 200 km. would lead to temperatures above the melting point. On the other hand, a distribution of radioactivity through the upper 200 km. of the mantle would require a much greater radioactive concentration than that found in ultra-basic rocks or stony meteorites. A deeper distribution of radioactive heat sources without producing melting would be possible if rocks at depth have a much higher thermal conductivity than is generally assumed, or by convection in the mantle.

It may be argued that the variations observed in the oceanic heat flow are due to convection currents in the mantle. Thus, for example, the recent high values found by Von Herzen[2] along the East Pacific Rise with areas of low heat flow flanking it may be due to a large convective cell in the upper part of the mantle. But if such an explanation is true, it is difficult to see why convection cells should be confined to the oceanic mantle and not be present under the continents, where the average heat-flow values show considerably less variation.

The concentration of uranium, thorium and potassium in the outer layers of the Earth must have occurred during its very early history (whether a hot or cold origin is postulated), the thickness of this outer layer being a few hundred km. The mantle under the oceans is thus presumably 'primeval', while that under the continents is 'anomalous'. However, the continents were formed, whether initially or whether, as advocated by Wilson[2], for example, they have grown from nuclei throughout geological time drawing their material from the mantle, the fact remains that the constitution of the upper part of the mantle is at present very different beneath the oceans and beneath the continents. It is this fundamental difference that makes it very difficult to see how continental drift can have occurred —unless the continents drag the upper few hundred km. of the mantle along with them, which seems extremely unlikely.

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Isotopic Distribution in a Greenland Iceberg

THE heavy-oxygen content in the ice of the Greenland ice cap is extremely low[1-3]. This is part of a common feature showing decreasing $H_2{}^{18}O$ content in fresh water when going towards a colder climate[1].

At a given location the seasonal variation in temperature causes a similar variation in the heavy-oxygen content of the precipitation[3,4]. On the Greenland ice cap, where the precipitation accumulates, this latter variation has been refound by investigation of stratigraphical series of snow and ice samples from a borehole 400 m. deep[3]. Parallelism between the deuterium and oxygen-18 content has been shown in snow samples from the Greenland ice cap[5]. The present work shows this parallelism to be conserved in the ice during several hundred years.

The samples investigated were collected during the North American Arctic Institute Greenland Expedition 1958 headed by Dr. P. F. Scholander. The deuterium analyses were made at Centre d'Etudes Nucléaires de Saclay by the technique described in ref. 6, while the oxygen-18 measurements were carried out at the University of Copenhagen by the technique described in ref. 7.

The samples were all taken from one piece of ice emitted from the Ingnerit glacier in West Greenland (70·9° N.). This piece was part of a larger amount of ice the average age of which has been determined by Scholander and co-workers by means of the carbon-14 method using the technique described in ref. 8. The piece of ice in question was white with mostly round bubbles. Three parallel layers of clear ice were situated 33 and 25 cm. from each other. The samples were taken along a line perpendicular to these layers.

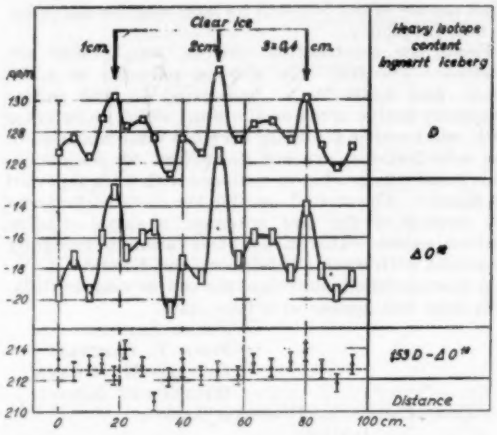

Fig. 1

In Fig. 1 the deuterium content (D) and the deviation of the oxygen-18 content (ΔO^{18}) of the sample from a standard are plotted in units of p.p.m. against the distance of the sample from an arbitrary zero point. In the lower part of Fig. 1 the relation between D and ΔO^{18} is seen to be linear within the measuring accuracy. With the use of our particular standard for oxygen-18 the linear relation is $1{\cdot}53\,D - \Delta O^{18} = 212{\cdot}7$. This shows complete parallelism between the variations in deuterium and oxygen-18, and, furthermore, conservation of isotopic anomalies in the ice during several hundred years.

The highest heavy isotope contents are found in the parallel layers of clear ice probably originating from summer-melting at the time of formation of the ice. This is due to evaporation from the wet snow; the light isotopic component of the water being the most volatile one.

We wish to thank Dr. P. F. Scholander and Dr. J. Koch for their interest in the work. The project was sponsored by the Arctic Institute of North America with principal support from the U.S. Office of Naval Research. Financial support was also granted by Statens almindelige Videnskabsfond, Copenhagen.

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PHYSICS

A Very General Class of Exact Solutions in Concentration-dependent Diffusion

THE mathematics of concentration-dependent diffusion has been found in recent years to be important in problems of diffusion in polymers[1-3] and in the problems of the transport of water in its various phases in soils and other porous materials[4-7]. Almost without exception, it has been necessary to use numerical methods to secure the necessary solutions[2,3,8,9]. According to Crank[10], the only known 'formal solutions' are due to Fujita[11,12]. They provide solutions of the equation :

$$\frac{\partial\theta}{\partial t} = \frac{\partial}{\partial x}\left(D(\theta)\frac{\partial\theta}{\partial x}\right) \qquad (1)$$

subject to the conditions :

$$\begin{aligned} &\theta = 0, \quad x > 0, \quad t = 0; \\ &\theta = 1, \quad x = 0, \quad t > 0; \end{aligned} \qquad (2)$$

and are for the following $D(\theta)$ functions :

$$D = D_0/(1 - \lambda\theta); \; D = D_0/(1 - \lambda\theta)^2; \; D = D_0/(1 + 2a\theta + b\theta^2).$$

Here we report the existence of a very general class of $D(\theta)$ functions which yield exact solutions of (1) subject to conditions (2). Fujita's $D(\theta)$'s are contained in this class. A similar very general class of $D(\theta)$ functions yields exact solutions of (1) subject to another set of practically important governing conditions:

$$\theta = 0, x > 0 \text{ and } \theta = 1, x < 0, t = 0;$$
$$\int_0^1 x \, d\theta = 0, t \geqslant 0. \qquad (3)$$

The substitution:

$$\varphi = xt^{-1/2}$$

enables (1) subject to (2) or (3) to be reduced to[8]:

$$\int_0^\theta \varphi d\theta = -2D \, d\theta/d\varphi.$$

That is:

$$D = -\tfrac{1}{2}\int_0^\theta \varphi \, d\theta \, . \, d\varphi/d\theta.$$

It follows that the solution of (1) subject to (2) or (3), $\varphi(\theta)$, will exist in exact form, so long as $D(\theta)$ is of the form:

$$D = -\tfrac{1}{2}\int_0^\theta F \, d\theta \, . \, dF/d\theta, \qquad (4)$$

where F is an analytical (I use this adjective to describe infinitely differentiable functions of real variables) function of θ which satisfies the following conditions: (*a*) the integral $\int_0^\theta F \, d\theta$ exists; (*b*) $dF/d\theta \leq 0$; (*c*) for (1) subject to (2), $F(1) = 0$; (*d*) for (1) subject to (3), $\int_0^1 F \, d\theta = 0$. (Note that D will be expressible in terms of known functions so long as F is integrable in terms of known functions.)

A very large class of F functions satisfies these conditions, so that, accordingly, there exists a very large class of D functions which lead to exact solutions of (1) subject to the appropriate conditions. There is thus an *embarras de richesse* of exact solutions available. A few very simple results are given in Tables 1 and 2.

Table 1. SOME SIMPLE CASES OF EXACT SOLUTIONS OF (1) SUBJECT TO (2)

D	φ	Remarks
$\frac{n}{2}\theta^n\left(1 - \frac{\theta^n}{1+n}\right)$	$1 - \theta^n$	$n > 0$
$\frac{n}{2}\theta^{-n}\left(\frac{\theta^{-n}}{1-n} - 1\right)$	$\theta^{-n} - 1$	$0 < n < 1$
$\frac{1}{2}\sin^2\frac{\pi\theta}{2}$	$\cos\frac{\pi\theta}{2}$	
$\frac{1}{2}e^{1-\theta}(1 - e^{-\theta} - \theta/e)$	$e^{1-\theta} - 1$	

Table 2. SOME SIMPLE CASES OF EXACT SOLUTIONS OF (1) SUBJECT TO (3)

D	φ	Remarks
$\frac{n(n+1)}{2}\theta^n(1 - \theta^n)$	$1 - (n+1)\theta^n$	$n > 0$
$\frac{n}{2(1+n)}[(1-2\theta)^{n-1} - (1-2\theta)^{2n}]$	$(1-2\theta)^n$	$n = 1, 3, 5, 7, \ldots$
$\frac{n}{2(1-n)}\theta^{-n}(\theta^{-n} - 1)$	$\theta^{-n} - \frac{1}{1-n}$	$0 < n < 1$
$\frac{e\theta}{2}[(e-1)\theta + 1 - e^\theta]$	$e - 1 - e^\theta$	

To this point we have developed these ideas under the restriction that F be analytical. However, the existence of D as a continuous function requires merely that F be differentiable once. Further, if a finite number of discontinuities in D is allowable, we may permit F to be not differentiable at a finite number of points in the θ-interval. That is, F may consist of a finite number of different analytical functions of θ, each covering a separate part of the θ-interval. The scope for using analytical methods is therefore even wider than might appear at first glance.

The range of exact solutions is so great that the $D(\theta)$ functions encountered in practical applications should be capable of accurate representation by the form (4). It would therefore seem that foundations have been provided for placing the theory and practice of much of the mathematics of concentration-dependent diffusion on an analytical basis. These matters are treated further in papers in the press in the *Australian Journal of Physics*.

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GEOLOGY

Presence of Carbohydrates and Other Organic Compounds in Ancient Sedimentary Rocks

Most investigators have been of the opinion that proteinaceous and carbohydrate materials that are deposited in marine and lake sediments cannot survive for long periods of time but are rapidly decomposed. However, in recent years several investigators have shown the presence of amino-acids in Tertiary shales[1] and in fossil shells and bones as old as the Devonian[2].

Preliminary studies have now demonstrated the presence of minute amounts of carbohydrate as well as proteinaceous substances in bituminous sedimentary rocks ranging in age from Ordovician to Tertiary. The reducing environment under which these sediments accumulated, coupled with the association between the organic compounds and finely divided inorganic matter, probably account for the preservation of these compounds.

Outcrop and well-core samples were thoroughly cleaned by immersion in chromic acid and subsequent washing with water. After being powdered, each sample (about 500 gm.) was treated with 0·5 *N* sulphuric acid for 8–10 hr. on a boiling water-bath. The hydrolysis mixture was neutralized with barium carbonate and freed from inorganic matter by ethanol precipitation and by ion-exchange resins. The hydrolysate from each sample was concentrated *in vacuo* and examined by paper chromatography. The following sugars were tentatively identified: glucose, xylose and arabinose, and possibly galactose and rhamnose (see Table 1). The concentration of glucose, which was the dominant component, expressed in mgm./kgm. of rock sample, varied from 0·14 for the Simpson to 1·3 for the Marcellus sample. In certain of the rock samples there was some indication of the presence of glycerol.

The glucose (180×10^6–300×10^6 years old), isolated from the Leonard and the Marcellus formations by the above treatment, was separated by paper chromatography (solvent: pyridine–ethyl acetate–water (2 : 5 : 7)) and characterized as the D-isomer by transforming it into N-*p*-nitrophenyl-β-D-glucosylamine, m.p. and mixed m.p. 184° C., $[\alpha]_D^{20} - 200°$ (approx.) in pyridine (*c.* 0·1)[3].

The mode of occurrence of the carbohydrate compounds in the rock formations is not yet known.

Table 1. Carbohydrate Components of Sedimentary Rocks

Formation	Age	Component sugars				
		Glucose	Galactose	Arabinose	Xylose	Rhamnose
Stonehenge	Ordovician	+		(? +)	(? +)	
Simpson	Ordovician	+	(? +)	+	+	
Upper Chambersburg	Ordovician	+		(? +)		
Marcellus	Devonian	+	(? +)	+	+	+
Woodford	Upper-Devonian	+		+	+	
Des Moines	Penn-sylvanian	+		+	+	
Missouri	Penn-sylvanian	+				(? +)
Leonard	Permian	+	(? +)		+	
Schuler	Jurassic	+		+	+	
Green River	Eocene	+	(? +)		(? +)	
Elko	Miocene	+				

The rock was treated with hot 0·5 *N* sulphuric acid and the sugars identified chromatographically.

It has been shown, however, that in the case of a sample from the Marcellus formation, extraction with water yielded about 10 per cent of the total carbohydrate material as the free sugars, arabinose, glucose and xylose; the remaining undissolved carbohydrate material may be present in the polymeric form.

The amino-acids present in the acid hydrolysates of several rock samples were also investigated by paper chromatography. In comparing two core samples of shale of late Palæozoic age, the highly fossiliferous one of Pennsylvanian age appeared to contain alanine, glycine, valine, glutamic acid, aspartic acid, proline, leucine, cystine and tyrosine, whereas the non-fossiliferous shale of Permian age yielded only one amino-acid tentatively identified as aspartic acid. The amino-acids in the Pennsylvanian sample may be derived from fossil shell fragments.

Control experiments showed that neither the sugars nor the amino-acids arose from the materials used in the isolation techniques. These organic substances are believed to be original constituents of the rocks, although in the outcrop samples, despite careful collection and cleaning treatment, the possibility of surface contamination cannot be completely ruled out at present.

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Evidence for Tertiary Crustal Distortion in Mid-Argyll

Regional deformation associated with the emplacement of Tertiary igneous rocks is well known in the British Isles. There appears to be some evidence in mid-Argyll, recorded on the one-inch maps of the Geological Survey of Scotland[1], which suggests that crustal distortion (broadly similar to that described from east Greenland by Wager and Deer[2]) may have occurred in this area during the Tertiary. This distortion is now reflected by a change in the trend of the Old Red Sandstone minor dyke swarm, the north-north-east–south-south-west tear-faults and, also, by variations in the strike of the Dalradian country-rocks; the district over which this change takes place coincides with the path of the Tertiary dyke swarm centred on Mull.

The Old Red Sandstone dyke swarm of the south-west Highlands has a north-north-east–south-south-west trend for most of its length[3]. However, just to the south of Loch Etive[1], a slight swing is seen and the dyke swarm traces out a gentle arcuate curve (Fig. 1). In the Craignish–Kilmartin area, twenty miles south-south-west of Loch Etive, the dykes have returned to their north-north-east–south-south-west trend; the greatest deflexion of the dykes from their normal orientation is about 15°. It is of importance

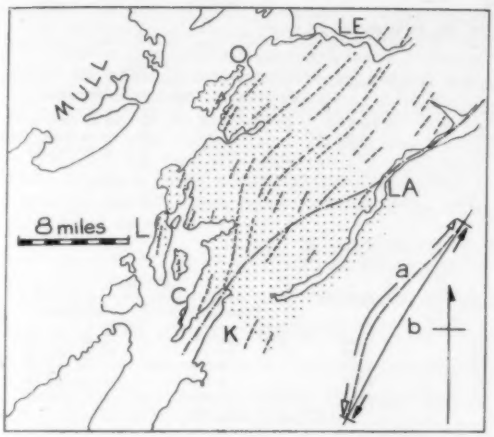

Fig. 1. Trend of Old Red Sandstone minor dykes (dashed lines) and Ericht–Craignish fault (alternately dotted and dashed line). Stippling represents the main zone of Tertiary dykes on the mid-Argyll mainland. The diagram in the lower right-hand corner illustrates the method of measurement of crustal extension (Extension = a–b/b). *LE*, Loch Etive; *O*, Oban; *LA*, Loch Awe; *C*, Craignish; *K*, Kilmartin; *L*, Luing

to note that the dyke swarm follows this arcuate trend, irrespective of whether the individual intrusions are emplaced into the Old Red Sandstone Lorne lavas or the Dalradian country-rocks. This same curving change in attitude is also shown by the Ericht–Laidon tear-fault which passes through Loch Awe to Loch Avich and Loch Craignish (Fig. 1); similarly, smaller tear-faults of the same set show a general arcuate swing. The strike of the Dalradian country-rocks and the axial trace of the Loch Awe syncline also appear to show the same change in trend (Fig. 2). It might be suggested that this flexure was developed in the Dalradian rocks during the Caledonian fold-movements and that the flexure then controlled the mode of emplacement of the later Old Red Sandstone dykes into both the Dalradian and the overlying Lorne lavas. This possibility appears unlikely because of the consistent north-north-east–south-south-west trend of the dyke swarm in the ground north-east of Oban[1]; in areas of Dalradian with variable strike,

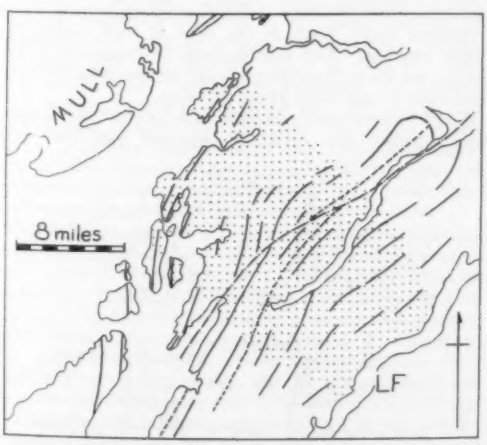

Fig. 2. Strike of the Dalradian country-rocks (solid lines) and axial trace of the Loch Awe Syncline (dashed line). Stippled area represents Mull Dyke Swarm. *LF*, Loch Fyne

there is no change in trend of the dykes. Similarly, the arcuate swing in the Ericht–Craignish fault and other sub-parallel faults cannot be readily explained except by later deformation.

It will be noted that (Figs. 1 and 2) the development of this arcuate structure is virtually restricted to the main zone occupied by the Mull Tertiary dyke swarm. The flexure appears to be dying out eastward and is scarcely recognizable near Loch Fyne (Fig. 2); there is, however, a slight curving in the Highland Boundary Fault where it is crossed by the Mull swarm, near Dunoon. Estimates of the crustal extension which might have been produced by the flexure can be made by comparing the distance along the arcuate trend of the flexure to that along the possible, undistorted trend: this is illustrated in Fig. 1. The estimate obtained in this way suggests a 3½ per cent extension between Luing and Oban and a 2½–3 per cent extension between Craignish and Loch Etive. These results can be compared to estimates, based upon actual measurements of dyke thicknesses, of a 4 per cent stretch along the south-east coast of Mull[2] and a 2½ per cent stretch along the western coast of Craignish[4].

I conclude that the coincidence in position between the arcuate flexure and the Mull dyke swarm is a strong indication that the flexure is the result of regional crustal distortion during the Tertiary.

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CHEMISTRY

Chain Transfer in Mixed Solvent Systems

Chain transfer for solvents[1–4] in addition polymerization has attracted much attention, but scarcely any attempt has been made so far to determine the chain-transferring capacities of those substances which are immiscible in the monomer (say, water) or substances insoluble in the monomer, for example, such polymers as polyacrylonitrile. In the present work an attempt has been made to determine the transfer constants for such substances, keeping the whole system homogeneous in the presence of a third component.

The general equation for the reciprocal average degree of polymerization in the catalysed system can be easily derived[4] as:

$$1/\bar{P} = C_M + C_S(S)/(M) + C_I(I)/(M) + \ldots (A^2/(M)^2) \ldots R_p \quad (1)$$

where $\bar{P}$ is the number average degree of polymerization, C_M, C_S and C_I are the chain-transfer constants for monomer, solvent and initiator respectively. (S), (M) and (I) are concentrations of solvent, monomer and initiator respectively, whereas R_p is the rate of polymerization. A is a constant and is the ratio of the termination (k_t) and propagation constant (k_p); that is to say, $k^{\frac{1}{2}}_t/k_p$ for termination by coupling and $2k^{\frac{1}{2}}_t/k_p$ for termination by disproportionation. For our present purpose we have determ-

Table 1*a*

S_1, *n*-butyl alcohol; $C_{S_1} = 1{\cdot}0 \times 10^{-4}$ (ref. 2); temperature, 60° C.

Solvent S_2	$M : S_1 : S_2$ (cm.³)	$10^5 R_p$ (moles/l./sec.)	$10^5 (1/\bar{P})$	$10^5 R_p A^2/M^2$	$10^4 C_{S_2}$	
					Present work	Literature
Carbon tetrachloride	2 : 1 : 1	2·07	38·72	14·88	19·90	15·81 (ref. 2)
Cumene	2 : 1 : 1	1·22	24·67	8·76	12·60	12·43 (ref. 7)*
Ethyl benzene	2 : 1 : 1	1·53	25·28	10·97	10·70	9·10 (ref. 7)

Table 1*b*

S_1, Methyl ethyl ketone; $C_{S_1} = 4{\cdot}5 \times 10^{-4}$ (ref. 2)

Solvent S_2	$M : S_1 : S_2$ (cm.³)	$10^5 R_p$ (mole/l./sec.)	$10^5 (1/\bar{P})$	$10^5 R_p A^2/M^2$	$10^4 C_{S_2}$	
					Present work	Literature
Carbon tetrachloride	1 : 1 : 1	2·46	19·95	8·08	18·30	15·81 (ref. 2)
Cumene	1 : 1 : 1	0·52	10·29	1·72	13·74	12·43 (ref. 7)
Ethyl benzene	1 : 1 : 1	2·02	12·72	6·66	6·72	9·10 (ref. 7)
Ethyl acetate	1 : 1 : 1	2·03	10·23	6·90	1·26	1·30 (ref. 2)
Cyclohexane	1 : 1 : 1	1·18	7·10	3·90	1·00	0·85 (ref. 7)

*Ref. 7, unpublished work in this laboratory.

ined A from the slope of the plot of $1/\bar{P}$ against R_p in the polymerization of methyl methacrylate initiated by 2,2′azo*bis*-*iso*butyronitrile.

In the case where two solvents S_1 and S_2 are employed the equation may be transformed to:

$$1/P = C_M + C_{S_1}(S_1)/(M) + C_{S_2}(S_2)/(M) + R_pA^2/(M)^2 \quad (2)$$

If azonitriles are used as initiators the C_I of which are well known to be approximately zero, this can be further simplified to:

$$1/P = C_M + C_{S_1}(S_1)/(M) + C_{S_2}(S_2)/(M) + R_pA^2/(M)^2 \quad (3)$$

Now if the value of C_{S_1} is known, this equation can be utilized to determine the value of C_{S_2} provided the whole system is kept homogeneous. In that case:

$$C_{S_2} = 1/(S_2)/(M) \ldots 1/P - C_M - C_{S_1}(S_1)/(M) - R_pA^2/(M)^2 \quad (4)$$

To check the validity of equation (4) and the method proposed we have utilized a number of solvents as S_2, the chain-transfer constants of which have been determined very carefully by previous workers[3]. A number of sets of experiments were carried out with methyl methacrylate at 60° C. *n*-Butyl alcohol and methyl ethyl ketone were chosen as solvents S_1. The value of C_M has been taken from our previous work[5], and $\bar{P}$ was determined by viscometric measurements using the Ostwald type of viscometer from the relation $\bar{P} = K(\eta)^\alpha$. K and α have been taken from the work of Baxendale *et al.*[6]. Polymerization experiments were carried out by the sealing-tube method already described[3,5].

From our results represented in Table 1 it can easily be seen that the values of C_{S_2} obtained by the present method compare favourably with values in the existing literature.

These results clearly show that the method suggested can be applied in evaluating chain-transfer constants for solvents. This opens up the possibilities of determining chain transfer for solvents immiscible with the monomer or in cases where the polymer separates out of the phase. Our present work relates to the chain-transferring capacity of water, the detailed results of which will be published in due course.

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Nuclear Spin Resonance Spectrum of Isotactic Polystyrene

The nuclear spin resonance spectrum of polystyrene has been reported by Bovey, Tiers and Filipovich[1]. Resonance peaks for *ortho* and for *meta* and *para* aromatic protons were observed. Absorptions by methylene and carbinyl (chain) protons appeared as a broader single peak in the case of high molecular weight species. For polystyrene of degree of polymerization 10 or less the carbinyl proton was resolved from that of the methylene protons.

We have now observed that carbinyl and methylene proton peaks are partially resolved also for high

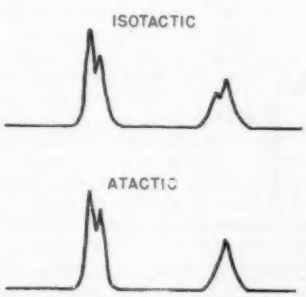

Fig. 1

molecular weight (10^5–10^6) isotactic polystyrene. The spectrum shown was obtained from isotactic polystyrene in deuterated chloroform using a 'Varian V–4300' 40·00-Mc./s. spectrometer. A similar spectrum was obtained in thionyl chloride. Like Bovey *et al.*, we observed no resolution of chain protons for commercial atactic polystyrene. Neither was resolution observed for high molecular weight (10^6) polystyrene prepared using *n*-butyl-lithium initiator nor for benzene-insoluble polystyrene obtained via Alfin sodium alkyl catalysis[2,3].

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Dissolution of Chrysotile Asbestos in Water, Acid and Alkali

The dissolution of the asbestos mineral, chrysotile, $(OH)_6Mg_6Si_4O_{11}$, has been studied as a part of investigations into the nature of the industrial lung disease, asbestosis[1]. Because the results may be of value to industrial chemists and to geologists, and they explain apparently anomalous results which have been published[2,3], they are outlined here although the details and a discussion on their relevance to the problem of pneumoconiosis will appear elsewhere.

At 85–90° C. chrysotile gives an extract containing appreciable amounts of monosilicic acid and magnesium, for example, 0·5 gm. chrysotile in 100 ml. water gives 0·4 mgm. magnesium and 0·2 mgm. *ortho*silicic acid per 100 ml. solution after 15 min. When extracted with water at 25° C. for some days, chrysotile gives a very low concentration of silicic acid in solution but more than ten times that concentration of magnesium. If the asbestos is removed and the extract is heated to 85–90° C., the concentration of the dissolved silicic acid increases more than ten-fold although the magnesium concentration is unaffected. As an example, after 5 days, 1 gm. chrysotile in 200 ml. water at 35° gave an extract containing 1·1 mgm. magnesium and 0·1 mgm. *ortho*silicic acid per 100 ml. After heating for 3 hr. at 90° C. the extract contained 1·2 mgm. magnesium and 1·4 mgm. *ortho*silicic acid per 100 ml. It is thought that magnesium ions are leached from the chrysotile giving fragments of the two-dimensional sheets of the silicon–oxygen lattice which are resistant to water except at elevated temperatures, when they break down to give *ortho*silicic acid.

Hydrochloric acid (0·1 *N*) attacks chrysotile giving a solution containing *ortho*silicic acid and magnesium ions. Sodium hydroxide (0·1 *N*) scarcely attacks chrysotile, presumably because of a protective coating of hydrated magnesium ions on the surface of the fibres. If acid-washed chrysotile is extracted with sodium hydroxide, silica passes rapidly into solution but very little magnesium; the acid removes the protective layer, allowing the disintegration of the next layer of the silicon–oxygen lattice by the alkali. Evidently the behaviour of chrysotile depends on its history, and it is particularly vulnerable to alternating acid and alkaline conditions.

This communication describes a part of a programme of research which is proceeding under the auspices of the Asbestosis Research Council.

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Clathrate Compounds in Chromatography

Partition and adsorption chromatography can be successfully applied for separation of mixtures of substances which differ in type and number of functional groups, or for separation of members of a homologous series. Less success was attained in separation of isomers. We therefore sought for a process which would be selective as regards the shape and size rather than the chemical properties of molecules.

The discovery of clathrate compounds suggested that inside their structure molecules of definite shape and size might be incorporated[1,2]. The first inorganic compound of that group was monoamminonickel cyanide[3,4], but many other complexes[5] of nickel, cobalt, manganese and iron with four basic nitrogen molecules and some anions around the metal ion followed. These compounds are said to be highly selective towards clathrate formation with organic molecules; and, more important, their selectivity depends rather on the shape of incorporated molecules than on their volume.

The selectivity, especially towards aromatic molecules, the small solubility of some complexes of that type, and other properties suggested the use of the formation of clathrate compounds for the separation of substances on the chromatographic column.

In preliminary experiments we filled the column with water-insoluble tetra-(4-methylpyridino)-nickel dithiocyanate prepared by the method of Schaeffer *et al.*[5]. Later, we found that another complex, prepared by using for precipitation a mixture of β- and γ-picoline and 2,6-lutidine instead of pure γ-picoline (so-called 3rd coal tar base fraction), has better properties.

One of the most important problems was to find an appropriate solvent composition for use as a mobile phase. We have used the mixture of γ-picoline or 3rd fraction with water containing some potassium thiocyanate.

To obtain high precision of results, we have used the chromato-polarographic method[6]. The compounds separated and incorporated were aromatic and aliphatic nitro-compounds.

In these conditions we have investigated the separation of isomeric nitrophenols, nitroanilines, chloronitrobenzenes and nitrotoluenes. In all instances quantitative separation of *ortho* from other isomers was obtained. The *ortho* isomers are found in the first fractions of eluate and can be very accurately determined polarographically. Using more concentrated solutions of potassium thiocyanate and 3rd fraction, *meta*- and *para*-isomers can be eluted. In some instances the mobile phase composition can be so chosen that it is possible to separate completely all three isomers in one process. We have used 2·5 *M* potassium thiocyanate in 10 per cent 3rd fraction

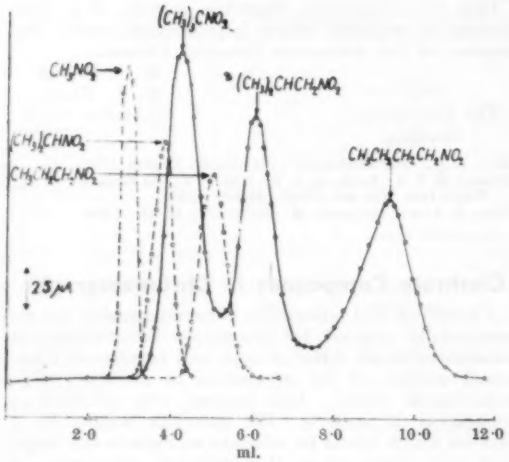

Fig. 1

base mixture for the separation of isomeric nitrophenols and nitroanilines (Kemula, W., Sybilska, D., and Geisler, J., unpublished work).

From nitroaliphatic compounds we have studied mixtures of 1-nitropropane and 2-nitropropane, 1-nitrobutane, 2-methyl-1-nitropropane and 2-methyl-2-nitropropane, but their separation is more difficult. No separation occurs when columns were filled with tetra-(4-methylpyridino)-nickel dithiocyanate or tetra-(3-methylpyridino)-nickel dithiocyanate. No insoluble complex could be prepared from pure 2,6-lutidine, but using the 3rd fraction base a very satisfactory column filling was obtained.

In Fig. 1 the continuous line indicates the separation of nitrobutanes, the dashed line indicates the elution of nitromethane and nitropropanes. The column height was 17 cm., its inner diameter 5·8 mm.; as an eluant we used 0·25 *M* potassium thiocyanate in 2 per cent 3rd fraction solution in water.

In Fig. 2 is shown for comparison the separation of the isomers using the reverse phase partition chromatography on powdered rubber[7]. As a stationary phase we used *n*-heptane, as a mobile phase 0·25 *M* potassium thiocyanate in 2 per cent 3rd fraction base mixture in water. The column height was 21·5 cm., the diameter 5·8 mm.

Our results show that the application of clathrate compounds in chromatography affords an excellent method for the separation of isomers. The mode of action of the complex prepared from 3rd fraction base mixture and the applicability of other complexes are being investigated. Such chromatographic studies may also be helpful in elucidating the properties of clathrate compounds and their structure.

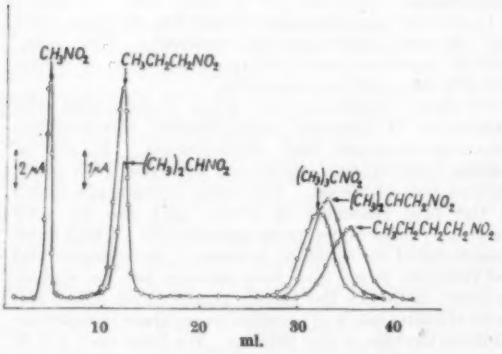

Fig. 2

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BIOCHEMISTRY

Casein labelled with Iodine-131 as a Substrate for the Measurement of Trypsin and Chymotrypsin

THE spectrophotometric method of Kunitz[1] is a marked improvement over previous methods for the measurement of trypsin and chymotrypsin activity. This procedure involves a measurement of the optical density at 280 mμ of the trichloracetic acid-soluble products formed during the enzyme hydrolysis of casein; it becomes impractical, however, where large concentrations of 280 mμ absorbing materials are present, such as in sera. In this communication, a modification of this procedure will be described which utilizes casein labelled with iodine-131 as the substrate and the measurement of radioactivity as an estimate of enzyme activity.

Labelled casein is prepared in the following manner. Into 12 ml. of 0·1 *M* disodium hydrogen phosphate–potassium dihydrogen phosphate buffer (*p*H 7·60) is placed 1 gm. of Hammarsten casein (Hammersten casein from Nutritional Biochemical Corporation); trypsin (50 per cent magnesium sulphate, 2 × crystallized) and chymotrypsin (salt-free, crystallized) from Worthington Biochemical Corporation; radioactive iodine from Abbott Laboratories, specify chemically pure product). The protein is stirred into solution while heating in a warm water-bath and then heated for 15 min. in a boiling water-bath. After cooling the solution to room temperature, the flask is placed on a magnetic stirrer and the casein iodinated with the following solution. Into 1 ml. of a sodium iodide solution containing 1 mc. of iodine-131 is pipetted the following reagents: 0·3 ml. 0·01 *M* potassium iodate, 0·3 ml. 0·01 *M* potassium iodate, drop by drop 0·05 *M* hydrochloric acid until the sodium iodide–iodine-131 mixture turns faint yellow, followed by 0·5 ml. of 0·05 *M* hydrochloric acid. The orange-coloured radioactive solution is mixed thoroughly and then added dropwise to the stirring casein solution over a period of 10–15 min. Stirring is maintained at room temperature for an additional 45 min. The iodinated casein is then transferred to a dialysis bag and dialysed against four 700-ml. portions of 0·1 *M* phosphate buffer (*p*H 7·35) in the refrigerator. The resulting labelled casein has a specific activity of 15–20 μc./ml. for the final casein solution and constitutes a 20–25 per cent radiometric yield.

The specific activity of free iodine in the fourth dialysate, which is also representative of the free iodine-131 contaminate of the labelled casein, is only on the average of 1·0 mμc./ml. This constitutes approximately 0·005 per cent of the total iodine-131 in the final casein solution.

Stock casein, unlabelled and un-iodinated, is used for making the appropriate dilution of the labelled substrate and is prepared as above. For enzyme measurement, a 2 per cent casein solution containing 1 μc. per ml. is prepared in 0·1 *M* phosphate buffer (*p*H 7·35).

Stock solutions of trypsin and chymotrypsin are prepared as 250 μgm./ml. in 0·0025 *M* hydrochloric acid. Diluted solutions of 25 μgm./ml. in 0·0025 *M* hydrochloric acid are further prepared for actual use.

Enzyme measurement is carried out as follows: Into 12-ml. centrifuge tubes are pipetted varying amounts of trypsin or chymotrypsin contained in less than 1 ml. volume. The volumes are made up to 1·0 ml. with 0·0025 *M* hydrochloric acid, mixed, and pre-incubated in a 37·5° C. constant-temperature bath for 5 min. The 2 per cent casein solution labelled with iodine-131 is pre-incubated at the same time. 1-ml. volumes of the casein solution are added with stirring to the assay tubes at 1-min. intervals. A blank tube containing 1 ml. of 0·0025 *M* hydrochloric acid is treated similarly. After a 20-min. incubation period, 3 ml. of 5 per cent trichloracetic acid are added with stirring to stop the enzyme action. The stirring rods are carefully removed and the solutions left at room temperature for 1 hr. Following a 20-min. centrifugation at 1,700*g* and 25° C., the supernatants are poured from the tubes. 1-ml. volumes of the supernatants are pipetted into 1-dram, screw cap vials and the radioactivity therein measured in a well scintillation detector.

In Fig. 1 is shown a typical plot of corrected counts (corrected for coincidence loss and for counts obtained in the blank tube) versus μgm. trypsin per ml. of the 1 per cent casein digestion mixture. Measurements

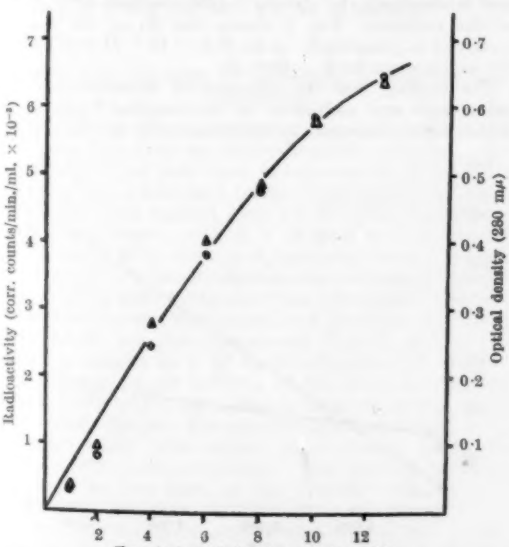

Fig. 1. Standard curve for digestion of casein labelled with iodine-131 by trypsin. Counts/min./ml. (circles) in trichloracetic acid supernatant and/or optical density (triangles) plotted versus micrograms of trypsin protein

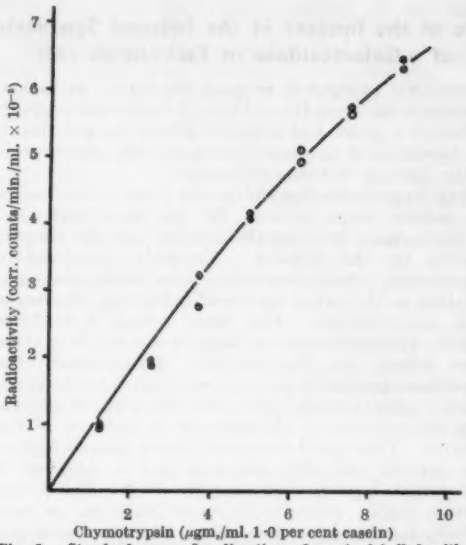

Fig. 2. Standard curve for digestion of casein labelled with iodine-131 by chymotrypsin. Counts/min./ml. in trichloracetic acid supernatant plotted versus micrograms of chymotrypsin protein

of the radioactivity in the supernatants have been found to correlate well with optical density measurements made at 280 mμ, as indicated in Fig. 1. Fig. 2 shows a similar curve obtained for the measurement of chymotrypsin activity by this method.

The use of a 2 per cent casein solution provides a linear relationship between enzyme activity and enzyme concentration over a greater range of enzyme concentration than is possible with the 1 per cent casein used by Kunitz[1]. Both 3 per cent and 4 per cent solutions serve to extend this linear relationship over a still further range of enzyme concentration. However, when using these more concentrated casein solutions, a relatively large amount of precipitate is obtained upon treatment with trichloracetic acid. Therefore, a correction should be made for the precipitates associated with each tube to obtain the correct curve representing enzyme activity.

This procedure utilizing labelled casein provides a sensitive means of measuring trypsin or chymotrypsin activity. Only those casein hydrolysis products which result directly from enzyme activity are measured. The spectrophotometric method could conceivably measure acid-soluble contaminants which absorb highly in the region of 280 mμ in addition to the casein hydrolysis products.

This method has been applied to the evaluation of the trypsin inhibitory capacity of sera of normal individuals and of patients in varying diseased states which are in part associated with altered trypsin metabolism.

This work was supported in part by the Charles F. Kettering Foundation, Yellow Springs, Ohio; the Montgomery County Society for Cancer Control, Dayton, Ohio; and the Ella Lowe Gunckle Fund.

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Role of the Inducer in the Induced Synthesis of β-Galactosidase in *Escherichia coli*

Induced (adaptive) enzyme formation in micro-organisms has been the subject of numerous studies[1]. Although a great deal is known about the kinetics of the biochemical processes involved, the precise role of the inducer remains enigmatic.

Any hypothesis describing the mode of action of the inducer must account for the structural complementariness between the inducer and the enzyme elicited by the inducer. Yudkin's 'mass action hypothesis'[2], which postulates inducer-enzyme combination as the prime mover of induction, represents such an attempt. The mass action hypothesis implies that substances capable of inducing an enzyme have affinity for the enzyme. Furthermore, the hypothesis predicts a positive correlation between the relative affinity of an inducer for the induced enzyme and the efficacy of the inducer as inducer of the enzyme. These predictions have been tested employing several inducible enzymes and a number of inducers in bacteria and yeasts[3]. It was found that certain highly effective inducers had no, or low, affinity for the induced enzyme. For this reason and also on the basis of certain other findings apparently incompatible with a mass action mechanism of enzyme induction, it was suggested that the inducer exerts its effect not by combining with the enzyme directly but rather by combination with some other cellular constituent. This hypothetical inducer-cell complex, the 'organizer'[4,5], would then catalyse formation of the enzyme.

One of the most thoroughly analysed inducible enzyme systems is that of the β-galactosidase of *Escherichia coli*. Monod and his collaborators[6,7] in a study of the relationship between the affinity of non-metabolizable galactosides for the enzyme β-galactosidase and the effectiveness of the same galactosides as inducers of the enzyme found that thiomethyl-β-D-galactoside and the α-galactoside melibiose were both highly effective inducers of β-galactosidase but had very low affinity for the enzyme. Affinity for β-galactosidase was determined by measuring the inhibition of the hydrolysis of the test substrate *o*-nitrophenyl-β-D-galactoside by the other two galactosides. A cell-free enzyme preparation was employed. It has been shown, however, that cell-free β-galactosidase (or the β-galactosidase of bacteria treated with toluene) differs from the intact-cell β-galactosidase with respect to its kinetic behaviour[8]. It has also been found more recently[9] that concomitant with β-galactosidase synthesis, the induced bacteria develop the ability to concentrate galactosides intracellularly. In view of these findings it was decided to re-examine the relationship between the affinity of galactosides for β-galactosidase and their inducing efficacy by (*a*) measuring the ability of galactosides to inhibit the β-galactosidase activity of intact cells and (*b*) determining the inducing efficacy of the same galactosides in *E. coli* strain *ML* 3, a strain that is unable to accumulate galactosides[9], and in which galactosides enter the cell apparently by diffusion only[10].

Fig. 1 shows that thiomethyl-β-D-galactoside and melibiose inhibit intact-cell β-galactosidase activity (measured as *o*-nitrophenyl-β-D-galactoside hydrolysis) competitively. Intact-cell β-galactosidase activity was measured in *E. coli* strain *ML* 308, which forms β-galactosidase constitutively and also has the

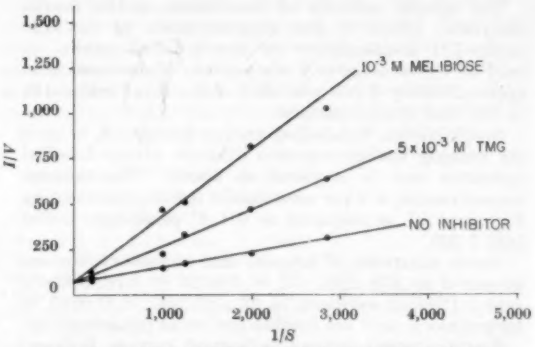

Fig. 1. Competitive inhibition of intact-cell β-galactosidase by thiomethyl-β-D-galactoside (TMG) and melibiose. Abscissa, reciprocal of *o*-nitrophenyl-β-D-galactoside concentration; ordinate, reciprocal of intact-cell β-galactosidase activity

ability to concentrate galactosides intracellularly[9]. The reaction was carried out as described in an earlier publication[11]. Michaelis constants of K_s $1{\cdot}3 \times 10^{-3}$ *M* *o*-nitrophenyl-β-D-galactoside and K_i $3{\cdot}3 \times 10^{-3}$ *M* thiomethyl-β-D-galactoside and $2{\cdot}5 \times 10^{-4}$ *M* melibiose were obtained. Thiomethyl-β-D-galactoside and melibiose do then show considerable affinity for the β-galactosidase of intact cells.

The affinity of an inducer for the hypothetical enzyme-forming system may also be expressed as a constant $K_{s\,\text{ind.}}$, where the latter term denotes that concentration of inducer which will induce a cellular concentration of enzyme that corresponds to half the maximal value obtainable, employing a saturating concentration of the same inducer and identical growth conditions. The $K_{s\,\text{ind.}}$ for thiomethyl-β-D-galactoside and melibiose were determined in *E. coli* strain *ML* 3 by growing the bacteria from an inoculum of approximately 10^6 bacteria per ml. to a final population of approximately 10^9 bacteria per ml. in the presence of a series of inducer concentrations and determining the specific β-galactosidase activities of the cultures. Fig. 2 shows the $K_{s\,\text{ind.}}$ for thiomethyl-β-D-galactoside to be $3{\cdot}5 \times 10^{-3}$ *M* and that for melibiose to be 2×10^{-4} *M*.

The finding that the affinities of thiomethyl-β-D-galactoside and melibiose for the enzyme β-galactosidase, when measured in the intact cell, are the same

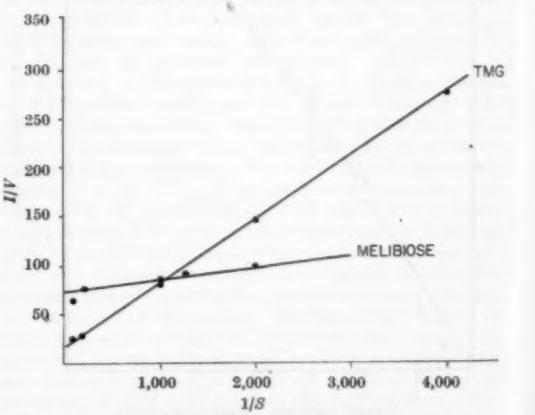

Fig. 2. Relation between the concentration of inducer present during growth and the specific β-galactosidase activity of the culture. Abscissa, reciprocal of inducer concentration; ordinate, reciprocal of specific β-galactosidase activity

as the affinities of thiomethyl-β-D-galactoside and melibiose for the enzyme-forming system is compatible with the hypothesis that enzyme induction involves combination between the inducer and the enzyme itself. The simplest scheme that would explain induced enzyme formation on this basis might be formulated thus:

$$X + \text{enzyme} + \text{inducer} \rightarrow X + \text{enzyme} - \text{inducer}$$
$$\text{enzyme} - \text{inducer} \rightleftharpoons \text{enzyme} + \text{inducer}$$

where X denotes the enzyme-forming system. Combination of the enzyme with the enzyme-forming system X in the absence of inducer might then constitute 'repression' of enzyme formation[12].

An amplification of the proposed hypothesis of induced enzyme formation, an attempt to relate the phenomenon of inducible galactoside accumulation to the induction of β-galactosidase, as well as further experimental data bearing on the hypothesis will be published elsewhere.

I am indebted to the National Science Foundation for a research grant and to Mrs. Helen M. Talbott for expert technical assistance.

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Ester-like Groups in Different Collagens and Gelatins

GALLOP *et al.*[1] have recently reported the presence of ester-like links in several soluble collagens. We had also found such links in commercial acid-precursor pigskin gelatin and bovine hide corium collagen.

Gelatin was treated with 1·5 *M* hydroxylamine at *p*H 8·6 in water, 5 *M* or 8 *M* urea or 5 *M* lithium bromide at 4° C. for 1 or 5 days or at room temperature for 4 hr. Bovine hide corium collagen (prepared by washing with water and 8 per cent sodium chloride and acetone) was first soaked in 4 *M* sodium perchlorate for 24 hr., the supernatant filtered off and the residue soaked in 3 *M* hydroxylamine (25 ml./gm. of collagen) at *p*H 8·65 for 16 hr. at room temperature. Almost all the collagen dissolved except for a few small shreds. The solution was dialysed at 4° C. against water with mixed ion-exchange resins in the external compartment. The protein solution gelled. 80 per cent of the original collagen was obtained as gelatin.

Analyses for hydroxamate were carried out on the pigskin product by the ferric hydroxamate method of Lipmann and Tuttle[3] (acethydroxamic acid as standard) and by the method of Bergmann and Segal[3]. The bovine collagen was analysed by the latter method.

The hydroxamate content of the acid pigskin gelatin varied from 0·5 to 1·5 moles/10^5 gm. The results were the same for all the methods of treatment mentioned above. The hide collagen had a hydroxamate content of 2 moles/10^5 gm.

The starting pigskin gelatin has a number average molecular weight of 100,000[4]. If one ester-like group were broken to yield two fragments of random size the average molecular weight would be 50,000. From measurements of intrinsic viscosity molecular weights of 25,000–35,000 were found, based on a viscosity–molecular weight relation established for this gelatin[4]. From melting points of 5 per cent gels, molecular weights of 30,000–70,000 were obtained, based on a melting point–molecular weight relation[4]. The discrepancy between the molecular weights by viscosity and melting point methods suggests that the reaction with hydroxylamine alters the shape of the gelatin molecules or changes the molecular weight distribution.

Hydroxamate values of 1–2 moles/10^5 gm. could be accounted for by carbohydrate; as little as 0·1 per cent would suffice. The differences between these values and the value of 10 moles/10^5 gm. found by Gallop *et al.* for soluble collagens suggest there may be differences related to whether the soluble or 'insoluble' collagen is used. There may also be differences resulting from species, tissue of origin and age of the collagen or the organism. Ageing organisms may contain increasing numbers of ester-like cross-links.

If water or sodium sulphate is used in place of sodium perchlorate, the collagen does not dissolve in hydroxylamine. Strong solutions of urea or sodium thiocyanate can be used in place of sodium perchlorate.

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Biosynthesis of Ergot Alkaloids

FIVE schemes for biosynthesis of the lysergic acid moiety of the ergot alkaloids have been proposed recently. All of them involve tryptophan or 5-hydroxytryptophan as precursors[1,2].

This communication deals with the effect of tryptophan, 5-hydroxytryptophan and its related compounds on the yield of total ergot alkaloids utilizing an *in vitro* culture of *Claviceps purpurea* to determine whether experimental evidence could be obtained to support one of the above schemes. A strain of *Claviceps purpurea*, *P.R.L.* 1578, was employed using Abe's medium[3].

Inoculation of the culture and the estimation of total alkaloids were carried out by a procedure slightly modified from that of Taber and Vining[4], and the composition of the total alkaloids was established by paper chromatography (ref. 5 and Weir, J. R., unpublished work). The R_F values of the spots found

Table 1. The Effect of Tryptophan and 5-Hydroxytryptophan on the Yield of Alkaloid Production

Age of cultures (day)		Additives	Mgm./l.	Mgm. alkaloid/l.		Deviation (per cent)
Feeding	Harvesting			Supplemented	Control	
11	23	DL-tryptophan	48·4	0·21	0·25	−16
11	36			2·77	1·99	+39
11	23	DL-5-hydroxy-tryptophan	32·8	0·21	0·25	−16
11	36			0·99	1·99	−50
22	29	DL-tryptophan	48·4	0·65	0·97	−33
22	43			2·17	3·27	−33
22	29	DL-5-hydroxy-tryptophan	32·8	0·61	0·97	−33
22	43			2·42	3·27	−26
30	51	DL-tryptophan	96·8	6·92	4·29	+61
30	51		242·0	6·60	4·29	+54
30	51	DL-5-hydroxy-tryptophan	90·4	5·75	4·29	+34
30	51		226·0	5·07	4·29	+18

were identical with the R_F values of pure ergosine, ergosinine or ergotaminine, ergokryptinine, elymoclavine.

Tryptophan, 5-hydroxytryptophan, indole, 5-hydroxyindole, serotonin were added to the cultures of different ages (11, 22, 30 days) and sampled after certain intervals (23, 29, 36, 43, 51 days).

Table 1 shows that addition of tryptophan or 5-hydroxytryptophan did not change significantly the yield of total alkaloids even if the amount of added DL-tryptophan or DL-5-hydroxytryptophan was gradually increased. An equivalent amount of indole, 5-hydroxyindole and serotonin also did not cause marked difference in the total alkaloid production.

In another experiment a replacement culture technique was used. On the thirty-ninth day, when alkaloid formation was 1·2 mgm./l. in the control culture, the growth medium was decanted off, the remaining mycelium was washed thoroughly with sterile water (3 × 20 ml.) and the following new media were added under sterile conditions: (*a*) 1 gm. mannitol in 20 ml. water; (*b*) 1 gm. mannitol, 0·160 gm. succinic acid in 20 ml. water; (*c*) 1 gm. mannitol, 0·160 gm. succinic acid, 0·0048 gm. DL-tryptophan in 20 ml. water; (*d*) 1 gm. mannitol, 0·160 gm. succinic acid, 0·0045 gm. DL-5-hydroxytryptophan in 20 ml. water; (*e*) 1 gm. mannitol, 0·160 gm. succinic acid, 0·0048 gm. DL-tryptophan, 0·0045 gm. L-proline, 0·0045 gm. L-valine, 0·0045 gm. L-leucine in 20 ml. water; (*f*) 1 gm. mannitol, 0·160 gm. succinic acid, 0·0045 gm. DL-5-hydroxytryptophan, 0·0045 gm. L-proline, 0·0045 gm. L-valine, 0·0045 gm. L-leucine in 20 ml. water.

After an incubation period of 19 days the total alkaloids were estimated. The results given in Table 2 indicate that there is no marked difference in the amount of total alkaloids in the replacement cultures and the cultures grown in the Abe's media harvested on the same day (control culture).

These experiments emphasize the significant role of the age of mycelium on the amount of alkaloid production of *Claviceps purpurea*. This observation seems to be supported by another experiment, when the medium of the 21-day-old culture (at this time no measurable amount of alkaloid was yet formed in the control culture) was inoculated with fresh mycelium. After harvesting the culture on the fifty-first day, the amount of total alkaloid was negligible although the weight of the mycelium was as usual.

Table 2. The Effect of Tryptophan, 5-Hydroxytryptophan on the Yield of Total Alkaloid Production in Replacement Cultures

Medium	Mgm. alkaloid/l.	
	Replacement culture	Control culture
a	4·61	5·07
b	4·28	5·07
c	4·47	5·07
d	4·54	5·07
e	4·86	5·07
f	5·11	5·07

Although all our experiments indicate that tryptophan and 5-hydroxytryptophan are not directly concerned in the biosynthesis of lysergic acid alkaloids, it cannot be concluded that these amino-acids are not the direct or indirect precursors of lysergic acid alkaloids. It is possible that the metabolism of these amino-acids is much faster in other directions than in the formation of alkaloids; therefore, they have no significant influence on the yield of alkaloid.

While this work was in progress, Suhadolnik *et al.*[6] reported that labelled DL-tryptophan was not incorporated into ergonovine produced by *Claviceps purpurea* cultured on rye. More recently, Taber and Vining[7] reported that large amounts of L-tryptophan (500 mgm./l. culture medium) doubled the amount of total ergot alkaloids formed by *Claviceps purpurea in vitro*. These authors also observed that the addition of L-tryptophan resulted in the production of new types of alkaloids and tryptamine. A direct comparison of the results obtained by Suhadolnik *et al.* and Taber and Vining with ours is difficult due to the different experimental conditions. (During the preparation of this communication an abstract of a paper appearing in the Bacteriological Proceedings, Fifty-ninth General Meeting, St. Louis, Missouri, was kindly submitted by W. A. Taber and L. C. Vining. Although no detailed results were given in this abstract it was stated that a culture of *C. purpurea* when supplied with tagged DL-tryptophan produced labelled lysergic acid derivatives.)

Use of the sensitive radioactive tracer technique is definitely indicated to obtain more specific information on the biogenesis of these alkaloids. This work is in progress and will be published in the near future.

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Glacial Acetic and Picric Acids as a Fixation for Gel Precipitation

Difficulties are often encountered in reading off immunological reactions on gel plates (cf. immunoelectrophoresis). The gel method, however, has many indisputable advantages over tube precipitation even if the latter is considered more sensitive quantitatively. The former has often been utilized for qualitative studies of the various components involved in immunological reactions. In this con-

nexion it is often important to know whether or not the plate contains a precipitate which is not visible either macroscopically or microscopically (cf. interference microscopy). Various fixatives have been employed in order to detect or accentuate an immunological reaction before proceeding to qualitative studies. Acetic acid and alcohol in various concentrations have been most commonly used for this purpose.

During work on an immunological system in which one could *a priori* expect a very weak precipitation reaction several fixatives were tested. These were chosen with consideration of their physical and chemical effects upon the various components in the system. It appeared that a combination of glacial acetic acid and saturated picric acid solution was preferable in those instances in which a quantitative reading of a precipitation reaction involving mainly proteins is desired.

The most satisfactory combination was found to be 6 per cent glacial acetic acid and 94 per cent saturated picric acid solution; pH = 2·1. In these proportions the penetrating effect of acetic acid and the protein-precipitating effect of picric acid appear to advantage. These acids seem to complement each other in other respects as well. (At this pH the protein-precipitating effect of picric acid is good and pH is not appreciably altered by the precipitating reaction.)

If this method is to be used when serum forms one of the immunological components, the serum must be highly diluted. If this is not done, the gel plate must be washed before being fixed (cf. agar gel with serum added) otherwise interpretation is made impossible by the large amount of precipitate originating from the other serum proteins.

In addition to its sensitivity, the method described here offers other advantages such as a short fixation time and a result which can easily be interpreted and recorded.

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Scanning of Paper Electrophoretograms after Protein Dyeing with Bromocresol Green

The blue colour of bromocresol green-dyed protein developed by exposure to ammonia vapour is not stable, and elution of the dyed protein has been used to obtain an electrophoretic curve from paper electrophoretograms of serum dyed with bromocresol green. One disadvantage of this is that the paper strip is destroyed when it would have been of value as a record for reference.

The use of an agent other than ammonia vapour was investigated with the view of stabilizing the blue colour of such dyed protein so that scanning could be carried out.

An organic base, with which the strip could be easily impregnated, strong enough to develop the blue colour of bromocresol green and which was miscible with the scanning oil, was looked for. Of several which were tried diethylamine was found to be suitable and the following method of use was found satisfactory.

The electrophoretograms were dried and then dyed according to the method described by Franglen and Martin[1]. The dry strips were then dipped in a solution of 10 per cent v/v solution of diethylamine in diethyl ether. The ether was allowed to evaporate and the blue colour of the bromocresol green-dyed protein developed. A series of dyed strips can be brought to this stage ready for scanning. The strips were then oiled for scanning using the mixture described by King and Wootton[2].

Scanning was carried out using an *E.E.L.* scanner with an Ilford 205 filter. After scanning, the oil and diethylamine were removed from the strips with either a 1 : 1 mixture of acetone and diethyl ether or benzene. The acetone used must be free from water otherwise diffusion of the dyed protein bands will occur. The strips were then air-dried ready for storage.

It was found that the blue colour remained stable for several weeks. This was probably due to the oil and diethylamine being removed by a non-ionic solvent leaving the bromocresol green in its blue form.

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Chromatography of Xanthene Dyes

Recently, Lima and Pieroni[1] have directed attention to the heterogeneous character of the dye rose bengal, which, when labelled with iodine-131, is used in tests of hepatic function[2,3]. Applying the ethanol–ammonia solvent system of Ishida *et al.*[4], they claim resolution of this dye into four components, three coloured and one colourless.

In the course of an investigation of the phloxin/tartrazine method, described by Lendrum[5] for the staining of connective tissues, I have had occasion to examine chromatographically most of the xanthene dyes in common use, and feel that my results may be of interest in relation to the observations quoted above.

A slight modification of the solvent system of Ishida *et al.*[4] was found to be the most useful; this consisted of 0·880 ammonia, ethanol and water in the proportion 5 : 10 : 85 (by volume). Paper chromatograms were run on Whatman No. 1 paper 20 × 40 cm. by the descending method of Consden *et al.*[6] with the modification that the dye was applied as a thin line at the starting point. Circular chromatograms by a variation[7] of the method of Rutter[8] were found to be convenient for the rapid comparison of different batches and gave good separations. R_F values tended to show some variation, and the values quoted below are the mean values of several results.

Commercial halogenated fluoresceins examined included eosin *Y* ([*R*]-tetrabromofluorescein), erythrosin *R* ([*R*]-di-iodofluorescein) and erythrosin *B* ([*R*]-tetraiodofluorescein). The first of these dyes showed two coloured components, R_F values 0·16 and 0·22; the second gave five components, R_F values 0·08, 0·15, 0·26, 0·49 and 0·64; the third dye gave two components with R_F values 0·08 and 0·15.

Studies made on the partial halogenation of fluorescein with bromine and iodine in aqueous alkali showed that even at the stage where only one molecular proportion of halogen had been added, the reaction mixture could be resolved chromatographic-

ally into five coloured components, the position of the absorption maxima of which decreased *pari passu* with increase of R_F value. The R_F values of these components were, after bromination: 0·16, 0·22, 0·33, 0·52 and 0·64; after iodination: 0·08, 0·15, 0·26, 0·49 and 0·64. The components with R_F 0·64 were identical and could not be distinguished on chromatographic or spectroscopic criteria from fluorescein. It would seem a reasonable assumption that the four other components in each case represent the mono-, di-, tri- and tetra-halogenated fluoresceins. Further halogenation of the initial mixture resulted in a gradual decrease in the proportion of components of high R_F value. The end-product in each case had two coloured components which appeared to be identical with those of commercial samples of eosin *Y* and erythrosin *B*, respectively.

Essentially similar results were obtained in experimental halogenation of [*P*]-tetrachlorofluorescein. The R_F values for the components were in this case: bromination: 0·40, 0·48, 0·59, 0·71 and 0·77 ([*P*]-tetrachlorofluorescein); iodination: 0·31, 0·42, 0·54, 0·71 and 0·77. A commercial sample of rose bengal (extra) had two coloured components with R_F values of 0·31 and 0·42; these were presumably the tri- and tetra-iodo[*R*]-tetrachloro[*P*]-fluoresceins. Six samples of commercial phloxin *B* (*C.I.* No. 778) were examined. These were generally more complex mixtures than the dyes mentioned above. Some of the fractions could be identified with the products of bromination of [*P*]-tetrachlorofluorescein and in one case there appeared to be admixture with iodinated fluoresceins. It is possible that the other components are derivatives of [*P*]-dichlorofluorescein, which is stated[9] to be the basis of phloxin (*C.I.* No. 774).

In addition to the multiplicity of coloured components in these dyes, an examination of the chromatograms in ultra-violet light revealed the presence of a number of colourless components which showed brilliant fluorescence. This was especially marked in the case of derivatives of [*P*]-halogenated fluorescein. In some of the samples of phloxin *B*, as many as four such components could be found.

In view of the statement by Stowe, Delprat and Weeks[10] that the liver only eliminates rose bengal when this latter is fully halogenated, a careful chromatographic examination would appear to be essential in this and similar applications of the xanthene dyes.

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PHYSIOLOGY

Hormonal Control of Moulting in Amphibians

MOULTING in amphibians starts when a new stratum corneum of the epidermis is formed. When moulting is normal the old stratum corneum is loosened and the slough thus formed is finally shed. (In the literature on amphibians, 'moulting' is often used synonymously with 'sloughing', that is, to denote merely the shedding of the slough.)

It is well known that moulting is abnormal in hypophysectomized amphibians. This is especially conspicuous in toads and most urodeles where hypophysectomy prevents shedding of the slough, whereas formation of strata cornea continues; eventually a dark-brown, thick, cornified layer covers the skin.

In urodeles thyroidectomy inhibits sloughing as effectively as does hypophysectomy. Reversely, sloughing can be produced by injection of thyrotropic hormone into hypophysectomized animals or of thyroxin into hypophysectomized or thyroidectomized animals[1–3]. Sloughing was also caused by thyroxin in hypophysectomized *Bufo arenarum*, but was not inhibited by thyroidectomy[4]. Sloughing was not inhibited by thyroidectomy or treatment with thiourea or thiouracil in *B. regularis* (Ewer, D. W., personal communication), or by treatment with thiourea or thiocyanate in *B. bufo* (Rosenkilde, Per, personal communication).

During investigations on hypophysial function in the toad *B. bufo* we observed that injection of adrenocorticotrophic hormone could cause sloughing in hypophysectomized animals. It was therefore found of interest to compare the effects of thyroxin and adrenocorticosteroids on moulting after hypophysectomy in a urodele (*Amblystoma mexicanum* Cope) and in a toad (*B. bufo* (L)).

Normally, *A. mexicanum* does not metamorphose. The skin of the unmetamorphosed animals does not hyperkeratinize in response to hypophysectomy in contrast to the skin of metamorphosed animals[5]. Metamorphosis was therefore induced by injection of 50 μgm. thyroxin. The animals of both species weighed around 50 gm. In *Bufo* only pars distalis of the hypophysis was extirpated; in *Amblystoma* the neuro-intermediate lobe was generally removed together with the pars distalis, because the two parts are not easily separated in this species. The technique of operation in *Bufo* has been described previously[5]. A similar technique was used in *Amblystoma*. Injections were given subcutaneously of thyroxin (Roche) and "Decorton" (Organon, deoxycorticosterone acetate, D.C.A., dissolved in peanut oil). Control injections of peanut oil were made.

In hypophysectomized *Amblystoma*, 50 μgm. thyroxin caused sloughing 2–3 days after injection, whereas in hypophysectomized *Bufo* 50 μgm. or 100 μgm. thyroxin had no effect (Table 1). In contrast, deoxycorticosterone acetate caused sloughing in *Bufo*, but not in *Amblystoma*. Both in *Amblystoma* and *Bufo* 0·5 mgm. deoxycorticosterone acetate was given twice a week for several weeks. The slough was not shed in *Bufo* after every injection, but when sloughing occurred it was always within 24 hr. after the injection. In seven toads 33 moults were produced; one toad died a week after hypophysectomy without sloughing. Control injections of oil had no effect on the skin. Mostly the sloughs were eaten

Table 1. EFFECT OF THYROXIN AND DEOXYCORTICOSTERONE ACETATE ON SHEDDING OF CORNIFIED LAYER OF EPIDERMIS IN HYPOPHYSECTOMIZED *Bufo bufo* AND *Amblystoma mexicanum*

Treatment	Species	Dose (mgm.)	No. of animals in which cornified layer was Shed	Not shed
Thyroxin	*B. bufo*	0·05	0	8
		0·1	0	6
	A. mexicanum	0·05	5	0
Deoxycorticosterone acetate	*B. bufo*	0·5	7	1
	A. mexicanum	0·5	0	5

as they are during normal moulting. However, the injections did not restore the normal moulting rhythm. New strata cornea were produced two or more times a week as in untreated hypophysectomized toads, whereas the normal controls moulted only about once a week.

It is probably not justified to conclude from the present experiments that sloughing is dependent exclusively on the thyroids in *Amblystoma* and on the adrenals in *Bufo*. Both glands may play a part in sloughing as well as other phases of moulting in both *Amblystoma* and *Bufo*, though seemingly to varying degrees.

In the literature it is more often tacitly understood than directly expressed that the moulting cycles in amphibians are maintained and controlled by rhythmic activity of the thyroids. Adams *et al.*[6], however, clearly state that they find it likely that the moulting rhythm in *Triturus* "is dependent at least in part on an endocrine (pituitary–thyroid) balance". Scharrer[7] considers the moulting rhythm in *Bufo* to be regulated by a special centre in the central nervous system, the centre acting via the hypophysis and the thyroids.

However, the following observations do not support the assumption that the single moult is normally initiated by increased activity of the thyroids—or other endocrine glands.

(1) In metamorphosed *Amblystoma* the transplanted hypophysis is able to maintain the thyroid activity at a level that permitted the normal moulting cycles to continue. The glands were transplanted under the palate mucosa and could therefore scarcely be rhythmically stimulated by any moulting centre[8].

(2) Injection of thyroxin into normal *Amblystoma* or *Bufo* did not immediately cause sloughing or loosening of the stratum corneum, but the moulting frequency was raised during the week or weeks following a single injection. This was especially pronounced in *Amblystoma*, which moulted almost daily for about a week—or some five times faster than normally—after a single injection of 50 μgm. thyroxin (Table 2). After about four moults in rapid succession sloughing stopped completely for up to three weeks. During this period the epidermis became covered by a thick cornified layer which was finally shed spontaneously; and an almost normal moulting rhythm was resumed. The observations

Table 2. NUMBER OF MOULTS PER WEEK IN FIVE *Amblystoma mexicanum*

	Before	After injection of 50 μgm. thyroxin (weeks)						
		1	2	3	4	5	6	7
Normal animals	6*	18	2	0	3	7	8	8
Hypophysectomized animals	0	21	0	0				

*Average of the seven weeks that preceded the injection.

are naturally explained by assuming that the high level of thyroxin in the blood immediately after the injection enhanced the rate of moulting, but at the same time inhibited the activity of the thyroids for several days so that the level of endogenous thyroid hormone did not permit sloughing. Shedding of the sloughs continued as long as sufficient exogenous thyroxin remained in the blood, and after this period did not start until the animal's own thyroids functioned again. In the hypophysectomized *Amblystoma* the single injection of thyroxin also initiated a burst of moults.

(3) Finally, it should be stressed that hypophysectomy inhibits the shedding, but not the formation of the slough; new strata cornea are still being formed and even faster than normally.

Thus normal moulting depends upon or may be influenced by the function of the thyroids, adrenal cortex and perhaps other endocrine glands as well[9]. The frequency of moulting is determined by the level of activity of the thyroids and not by a rhythm of activity; the ability to moult rhythmically is apparently inherent in the skin.

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Selective Biological Membrane Transport of Individual Amino-Acids

THE behaviour of amino-acids in high-tension (100–300 V./cm.) electrostatic fields has recently been investigated in this laboratory. A phenomenon (to be described shortly) was observed, and it led to a hypothesis which is summarized in the following.

Since the Donnan effect cannot explain the selectivity of membrane transport of molecules larger than mono- or di-atomic ions and the Michaelis theory[1] becomes more and more[2] justified, we think our observations have given us an explanation of many problems, among them, for example, why cells put into a concentrated solution of amino-acids take up only a determined amount of amino-acid and not more[3]. How can it be explained by the Donnan effect why cells do take up only specified amino-acids or other molecules, and why can one or the other be substituted by a completely different kind of molecule[4], etc.?

However, to-day we know more about the structure of some biologically important membranes. The electron microscope has shown that phospholipid molecules are arranged[5] in the anterior pituitary gland cell wall in a laminary way, 20 Å. apart from each other in triplets, making up a thickness of 75 Å. Halpern and Binaghi have shown a selective permeability of certain biological membranes[3] to carbonic acid, and a similarly selective activity of the human erythrocyte membrane towards uric acid has

been demonstrated[6], and further that calcium ion has an important role in maintaining this selective permeability was shown by Maizels[7]. Putting together the findings of the above workers and several others as well as ours we came to the following conclusion.

An amino-acid—let us keep for the sake of simplicity to this example—which has been liberated from the food protein by digestive ferments finally acquires an electrostatic charge in the jejunum. The amino-acid will then approach the wall of the gut (and it must not get nearer to it than 50 Å. [8]) at a point where there is a venous capillary or lymph duct on the other side. At these points the inside of the gut membrane will have a polarity and potential sufficiently great enough to attract the amino-acid, which bears an opposite polarity and has enough reserve energy[9] to alter its structure into another form—an elongated one most probably. This enables it to pass now the approximately 20-Å. wide opening on the membrane towards which it is pulled. As soon as the amino-acid reaches the other side of the membrane it will be pushed away since this side has the same polarity as the amino-acid, and will travel along by now, let us say, in the venous capillary, returning to its original structure. Since the *p*H value of the blood will differ from that in the gut, the amino-acid receives a new charge and also, possibly, alters its polarity.

The electrostatic structure of the ribo-nucleus molecule in a given cell, at some distance from our amino-acid, will cause the production by that cell of a certain quadruple peptide. When three given amino-acids in this cell are already linked together, a certain pattern of polarity and electrostatic charge will be set up, which will affect the cell membrane either as a whole or only a part of it. It is necessary for this hypothesis that this individual cell membrane should be some ten times more sensitive to changes in electrostatic charge than the gut membrane. This will enable the cell to discriminate between individual amino-acids. When the 'missing' amino-acid reaches the vicinity of the cell it will be attracted to it and will enter into it with the same type of changes as were described when it passed through the gut membrane, except that when it now reaches the cell interior, instead of moving around freely it will immediately combine with the already coupled group of amino-acids, thereby changing the electrostatic state of the cell again. The completed quadruple peptide will then be either incorporated structurally into the cell, or the polar and electrostatic changes will enable the peptide to pass through the cell membrane and get into the blood circulation, where it can be used up for any further macro-molecular building.

This selectivity of membrane transport for amino-acids has been demonstrated in this laboratory on a small-scale, simple model-experiment. Two amino-acids, histidine and aspartic acid, were dissolved in a buffer solution at a *p*H of 8·6 and kept at 38° C. with a constant slow stirring. Into it, five tubes were dipped, the lower end of each being covered by a 'Cellophane' membrane. The tubes contained the same buffer solution but the *p*H was varied in steps of 0·2 from 7·0 to 7·8. At the end of 5 hr. it was found that all aspartic acid was in the tube of *p*H 7·0 and all the histidine in the tube of *p*H 7·2. No amino-acid was detected in any of the other three tubes, or in the original buffer solution.

Further work on this problem is being carried out with the close co-operation of the Marconi Instruments Co., Ltd., on a larger and more critical scale. It is hoped that this further stage of the model-experiment will throw more light on the problem of specific membrane transport.

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Induction of a Vascular Permeability Factor by the Interaction of Guinea Pig Tissue Debris with Serum

The sera of the guinea pig[1,2], rat[3], rabbit[3] and man[4] contain the inactive precursor (*pro-PF*) of a globulin permeability factor (*PF*) activable by dilution in saline (*PF/Dil*) or by chemical fractionation. The *in vivo* mechanism of activation is unknown; but an obvious hypothesis—that activation in injury is induced by contact of the *pro-PF* with damaged tissue—was investigated by testing the effect on guinea pig serum of debris from homogenates of guinea pig tissues.

Guinea pig serum was harvested from defibrinated blood[5] and stored at 2° C. The excised tissues were freed of adherent connective tissue bearing blood vessels and disrupted[6] in sterile 0·85 per cent saline, using a loosely fitting homogenizer of the Potter type. The homogenate was centrifuged at 3,000*g* for 20 min. and the supernatant decanted. The residual tissue debris, twice washed in 20 vol. saline, was suspended in saline, such that 1 gm. wet tissue yielded 1·5 ml. tissue debris suspension. Dried tissue debris was obtained by adding the suspension to 10 vol. acetone, removing the debris 10 min. later by filtration and drying it in a stream of air. Permeability-increasing activity of the preparations was estimated in terms of the diameter of the area of exudation of circulating pontamine blue induced at the site of intracutaneous injection in depilated guinea pigs[7].

The ready and progressive activation of *pro-PF* to *PF/Dil* by dilution of normal serum in saline depends on the age and temperature of the dilutions[1]. In preparations held 90 min. at room temperature, maximum permeability-increasing activity occurs in 1/150 serum, but at this time low dilutions of serum, 1/1–1/15, have no substantial activity because activated *PF* has been antagonized by a low-potency, slowly acting inhibitor (*IPF*) present therein. A second factor (*PF/Age*)[1] matures in serum kept for one or more days. *PF/Dil* is characterized by its insusceptibility to the antihistamine, mepyramine maleate, and its high susceptibility to trypsin inhibitors like that from soya bean (*SBTI*); *PF/Age* is not antagonized by *IPF*, *SBTI* or mepyramine.

Tissue debris suspensions of lung, liver, heart, skin, brain and skeletal muscle each were mixed with 2·5 vol. serum and after 15 min. the debris was removed by centrifugation. All except skeletal muscle induced permeability-increasing activity in the treated serum (Table 1). The most active, brain debris, was investi-

Table 1. PERMEABILITY-INCREASING ACTIVITY OF SERUM TREATED WITH HOMOGENATES OF VARIOUS TISSUES

Tissue homogenate	Mean diameter (mm.) of area of increased permeability induced by: Saline extract	Treated serum (1/3)
Lung	2·9	5·5
Liver	2·6	5·6
Heart	2·8	6·1
Skin	2·9	6·6
Brain	3·5	7·8
Skeletal muscle	3·9	3·9
None	—	4·2

gated. Mixed with normal serum, within 5 min. it led to the appearance of a new permeability factor in the 1/1–1/15 dilutions. The amount of factor produced was proportional to the amount of brain debris added; and 1 hr. exposure to debris was no more effective than 5 min. The new factor produced by the reaction of debris with serum differed from *PF/Dil* in being insusceptible to *SBTI*, only slightly susceptible to serum fractions containing *IPF*, but highly susceptible to mepyramine. In fact, the addition of mepyramine to dilutions of treated serum restored the pattern of activity to that of untreated serum. These results also distinguish the new permeability factor from *PF/Age*.

Saline extracts of dried brain debris and the supernatant fluid from brain homogenate also contain a permeability factor with the same susceptibility to inhibitors. Moreover, all three preparations—fresh debris mixed with serum, the supernatant from fresh debris and a saline extract of dried debris—induced a slow contractor response in atropinized guinea pig ileum *in vitro*, which was not suppressed by mepyramine; and on injection into the rat peritoneal cavity[8], all three readily released histamine (Table 2). That is, the new factor appears to be a histamine-liberator. Prepared from saline extracts of dried debris, it is stable for 10 min. at 60°, 80° or 100° C. It does not appear to be associated with a protease system (for example, plasmin) because *SBTI*, 333 μgm./ml., also fails to inhibit its appearance in mixtures of serum and debris.

Table 2. HISTAMINE RELEASED IN THE RAT BY THE INTRAPERITONEAL INJECTION OF DEBRIS FACTOR AND OTHER PREPARATIONS

Substance injected	Histamine yield Exudate (μgm./ml.)	Body-weight (μgm./100 gm.)
Compound 48/80 (ref. 11) 15 μgm./ml.	0·20	1·03
Debris in 1/3 serum	0·24	0·94
Untreated 1/3 serum	0·00	0·00
Supernatant of brain homogenate*	0·15	1·00
Dried debris	0·13	0·42
Locke's solution	0·03	0·14

* Prepared in Locke's solution.

It is suggested that many tissues normally contain a bound histamine-liberator, which is released on mechanical disruption, after treatment with acetone or by the action of serum on damaged tissue. Moreover, the results provide a model for a tissue mechanism of histamine liberation—serum releasing histamine directly from injured tissue, or activating the factor, which in turn liberates histamine. *In vitro* the reactions are rapid; release of the factor by serum is complete in 5 min., the shortest period tested; and the released factor, injected intracutaneously, increases permeability in ½ min.

The new factor may mediate the immediate permeability response of tissues to injury[9]. However, it is not possible at this stage to evaluate the relation of this system to the superficially similar one described by Spector[10] in the serum and pulmonary mitochondria of the rat, and ascribed by him to an activation of serum *pro-PF* by mitochondria.

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Ataxia in Hens poisoned by Tri*para*ethylphenyl Phosphate

IT has been known for almost 30 years that the administration of tri*ortho*cresyl phosphate and certain other organo-phosphorus compounds produces demyelination in the central and peripheral nervous system of some species. Earl and Thompson[1] suggested that the lesions might be associated with the anticholinesterase activity of the drugs *in vivo*, but Davison[2] later demonstrated that some other organo-phosphorus compounds, which were equally effective as inhibitors of cholinesterase, did not cause demyelination or ataxia. More recently another arylphosphate, tri*para*ethylphenyl phosphate, has been synthesized, which causes ataxia in hens[3], but, unlike the other demyelinating agents of this class, it does not possess demonstrable anticholinesterase activity (Aldridge, W. N., and Barnes, J. M., personal communication). The results with this material appear to provide conclusive evidence that the organo-phosphorus compounds do not produce demyelination by virtue of their anticholinesterase effects.

However, it may be wrong to assume that the toxic action of all the demyelinating drugs in this group is exactly the same. In this laboratory, it has been observed that the clinical picture produced in hens dosed with tri*para*ethylphenyl phosphate differs very strikingly from that seen with tri*ortho*cresyl phosphate poisoning. In hens poisoned by tri*ortho*cresyl phosphate, ataxia is associated with very marked flexion of the joints of the leg and with curling of the toes. In contrast to this, extensor tone is greatly increased in the hens dosed with tri*para*ethylphenyl phosphate; these stand on tiptoe and walk with exaggerated extension of the legs. In the late stages, when their legs are unable to support their weight, the birds sit with their legs extended rather than flexed. Another difference is in the time course of the condition; in tri*ortho*cresyl phosphate poisoning changes in gait are never seen before the seventh day after dosing, but once symptoms appear the condition progresses rapidly and many birds became moribund during the third or fourth week. Only those birds with relatively mild symptoms survive, and recovery is extremely slow; for example, one bird was still ataxic 27 weeks after dosing. On

the other hand, symptoms appear as early as the fourth day after tri*para*ethylphenyl phosphate has been given but they increase more slowly and less steadily than in tri*ortho*cresyl phosphate poisoning. While some of the birds with severe disabilities have died, others have shown obvious improvement from the fourth to fifth week and in some less-affected hens recovery has been almost complete 7 weeks after dosing.

Although no claim is made on the basis of this work that tri*para*ethylphenyl and tri*ortho*cresyl phosphates produce their effects by a different biochemical pathway, the findings suggest that such a possibility should be considered. In view of this it seems important that workers in this field should describe their clinical observations more precisely than hitherto. It may be wrong to classify all organo-phosphorus-produced disabilities as 'Ginger Jake paralysis'.

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Glucagon : a Protein Catabolic Hormone in the Isolated Perfused Rat Liver

WE have reported that the isolated perfused rat liver from a fasted animal produces urea in linear fashion[1], at the expense of both liver and plasma proteins. Urea production occurs, and the blood amino-acid level is maintained in the absence of added extraneous amino-acids. When a free amino-acid supplement is added, urea production is greatly enhanced. The addition of glucose or fructose supplements is associated with a nitrogen-sparing effect manifest as a diminished production of urea. This nitrogen-sparing effect is seen in either the presence or absence of added amino-acids.

The production of urea by the isolated perfused liver may be regarded as a measure of the continuing or endogenous protein catabolism, and the isolated perfused liver affords a means of studying protein catabolism and the effects of various agents upon it.

This communication reports a remarkable effect of the hormone glucagon (generously supplied by the Eli Lilly Co.) in enhancing protein catabolism in the isolated perfused liver.

In typical experiments a 6-hr. liver perfusion is started and continued for a base-line period of 1½–2 hr. During this time, Ringer's solution is continuously infused. After this the continuous infusion is changed to one of Ringer's solution containing glucagon in such concentration that as little as 1 μgm. of glucagon is infused per hour.

Blood samples are removed at regular intervals, analysed for urea by Conway's method and for glucose by Nelson's method. The total amount of urea produced during each interval is computed after appropriate correction for the amount of urea removed by the sampling procedure itself.

Not only is prompt glycogenolysis produced by as little as 1 μgm. of glucagon per hour, but also, as Fig. 1 shows, the rate of urea production is greatly increased. Almost complete glycogenolysis occurs in the face of blood glucose-levels known to promote glycogen deposition[2] and also to spare protein[1].

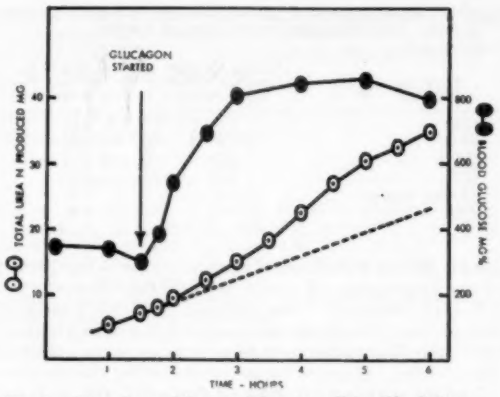

Fig. 1. Liver donor fed stock laboratory diet. Blood donors fasted 18 hr. before being bled to provide a total of 63 ml. of whole blood diluted with 35 mgm. heparin and Ringer's solution to 79 ml. at start of perfusion. Perfused liver weight, 14 gm.; glycogen content at 1½ hr. 2·64 gm. per cent, and at 6 hr. 0·11 gm. per cent. Over an interval of 1½–6 hr. glucagon was infused at the rate of 1 μgm. per hr. in 3 ml. of Ringer's solution

It is of some interest that the hormone hydrocortisone in the form of the soluble succinate given by continuous infusion in amounts up to 2 mgm. per hour produces no change in the output of urea by livers from either normal or adrenalectomized rats. Only when glucagon is infused into livers from the latter along with hydrocortisone is a maximal increase in protein catabolism elicited. Given alone, in perfusion of the adrenalectomized rat liver, glucagon causes a small but definite increase in protein catabolism.

The glycogenolytic effect of glucagon at 1 μgm. per hour on the liver is not altered by prior and continuous administration of insulin in amounts known to be sufficient to enhance glucose uptake and lipogenesis[3]. However, the protein catabolic effect of 1 μgm. per hour is abolished by the insulin.

Although relatively massive doses of glucagon given parenterally to intact rats have been associated with enhanced urinary nitrogen excretion[4], the site of action and the mechanism of the response have not been defined. Our experiments implicate the liver directly and point to glucagon as being a protein catabolic hormone *par excellence*, with adrenal corticoid hormone exerting a permissive action on this effect.

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Effects of Reserpine, Serotonin and Vasopressin on the Survival of Cold-Stressed Rats

In the preparatory stages of a projected study of the hypothalamic control of release of adrenocorticotrophic hormone, large doses of reserpine, serotonin creatinine sulphate, and commercial vasopressin were administered to rats which were then subjected to cold stress. Preliminary findings are reported here in view of the widespread interest in the drugs from the point of view of pituitary-adrenal physiology[1] and in view of the current extensive use of reserpine in medicine.

Intact male rats of the Harvard strain weighing 95–105 gm. were selected. All substances were administered subcutaneously, and cold-exposed controls received volumes of vehicle equal to those injected into the test animals. The rats were placed in individual screen-bottomed cages without food or water in a cold room at 3° ± 1° C. either immediately following the injection, or in some cases 18 hr. after injection. To minimize variations due to cyclic changes in physiological activity the animals were placed in the cold room at approximately the same time (1200 midnight ± ½ hr.) except for one stressed control group which was exposed to cold starting at 11 a.m. Treated controls were kept at room temperature under otherwise similar conditions. For the sake of comparison a group of adrenalectomized animals (1 week after surgery) in the same weight range was similarly stressed. The results are summarized in Table 1.

It is evident that large doses of reserpine, serotonin creatinine sulphate, and commercial vasopressin effectively lower the ability of intact rats to withstand cold stress, although on the basis of the experiments described here it is not possible to offer any demonstrable explanation for the mechanisms involved; however, the similarity in the survival-times of serotonin- and vasopressin-treated rats and the survival times of adrenalectomized animals suggests that treatment with these two drugs—both of which are known to exert some action on the pituitary–adrenal system[1]—results in temporary functional adrenalectomy.

Table 1. Effects of Reserpine, Serotonin Creatinine Sulphate and Vasopressin on the Survival-Times of Cold-Stressed Rats

Treatment	Survival time*	No. of animals
1 Intact controls, exposed at 1200 midnight	19·25 ± 1·8 hr.	20
2 Intact controls, exposed at 11 a.m.	9·56 ± 1·0 hr.	9
3 Adrenalectomized, untreated animals	2·98 ± 0·8 hr.	9
4 2 mgm. serotonin creatinine sulphate, stressed immediately after injection	< 4 hr.	10
5 1 mgm. serotonin creatine sulphate, stressed immediately after injection	< 4 hr.	10
6 1 mgm. serotonin creatinine sulphate, stressed immediately after injection	3·57 ± 0·6 hr.	7
7 2 mgm. serotonin creatinine sulphate, stressed 18 hr. after injection	killed after 15 hr.	8
8 1 mgm. serotonin creatinine sulphate, stressed 18 hr. after injection	killed after 15 hr.	8
9 1 mgm. reserpine, stressed immediately after injection	9·5 ± 0·6 hr.	18
10 1 mgm. reserpine, stressed 18 hr. after injection	10·5 ± 0·7 hr.	10
11 3 units vasopressin, stressed immediately after injection	2·08 ± 0·15 hr.	6
12 2 units vasopressin, stressed immediately after injection	3·06 ± 0·53 hr.	9
13 3 units vasopressin, stressed 18 hr. after injection	killed after 15 hr.	6
14 2 units vasopressin, stressed 18 hr. after injection	killed after 15 hr.	6

All treated controls kept at room temperature survived for 24 hr. and were killed at that time.
* Mean ± standard error of the mean.

Reserpine clearly differs from the other substances tested in that its effects are still evident 18 hr. after injection, although most of the drug is known to have been metabolized by then[2]. The survival times of intact controls exposed to cold stress at different times of the day appear to reflect the daily cyclic changes in adrenal activity[3].

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Potency of Parenteral Vitamin A

Vitamin A is widely employed in clinical practice and is also used extensively as a teratogenic agent for the production and study of fœtal abnormalities in laboratory animals[1].

Up to the present the customary procedure in experimental work has been for the vitamin to be dissolved in oil and administered orally. This method has a number of disadvantages. In order to ensure that the animal receives the required dose, it is necessary to put the dissolved vitamin directly into the stomach by the somewhat laborious procedure of gastric intubation. Once in the alimentary tract, defects of absorption may lead to the loss of a substantial amount of the vitamin in the fæces. Such defective absorption may be due to the presence of gastric or intestinal inflammatory conditions, to a deficiency in the secretion of bile which leads to a failure in the breakdown of the fat in which the vitamin is dissolved, or to the nature of the oil itself[2]. Some of these factors may also militate against absorption of the vitamin when employed for therapeutic purposes in man.

Subcutaneous or intramuscular injection has many advantages in experimental work from the point of view of convenience and the more precise measurement of dosage, but the results of the injection of oily solutions of vitamin A have been disappointing. Brown and Sturtevant[3] found that during a 28-day test period little if any benefit resulted from injection and concluded that vitamin A in oil injected subcutaneously or intramuscularly was utilized to a very small extent if at all. Their conclusions are borne out by the results of experiments which we have carried out in rats. The subcutaneous injection into rats of 60,000 I.U. vitamin A dissolved in arachis oil daily from the eighth to the thirteenth day inclusive of pregnancy did not produce any cleft palates in the young, whereas the same quantities given by gastric intubation over the same period of time resulted in 29·7 per cent of cleft palates in the young[4].

Recently we have had the opportunity of studying the teratogenic effects of the subcutaneous injection of vitamin A stabilized in 'Tween 80'. Female rats of the Wistar strain were used. After mating with normal male rats, pregnancy was determined by the presence of spermatozoa in the vagina. The pregnant females received 60,000 I.U. vitamin A (suspended in 'Tween 80') daily by subcutaneous injection on the twelfth, thirteenth and fourteenth days of pregnancy.

The rats were killed on the twentieth day of pregnancy and the fœtuses removed from the uteri for examination. The results of the two pilot experiments in relation to malformations of the palate are shown in Table 1. In addition to the palatal deficiencies many of the young showed other skeletal malformations involving mainly reduction of the digits and syndactly.

Table 1. INCIDENCE OF CLEFT PALATE FOLLOWING PARENTERAL VITAMIN A

Group	No. of dams	Total No. of young	No. of young with cleft palates	Percentage of young with cleft palates
1	8	68	49	72·0
2	7	54	49	90·7

The percentage incidence, in the fœtuses, of cleft palates produced by the subcutaneous injection of vitamin A compares closely with that of 92 per cent observed by Giroud and Martinet[5] when female rats were given 60,000 I.U. vitamin A, in oily solution, by mouth on the eleventh, twelfth and thirteenth days of pregnancy. The disparity in the percentage incidence of the malformation in our two groups of animals is probably due to the small numbers used in these pilot experiments, although some other unknown factor may be involved.

These experiments indicate that vitamin A suspended in 'Tween 80' is potent as a teratogen when injected subcutaneously. From the experimental point of view, the advantages of this route of administration over the oral are obvious. It may also be useful, at times, to be able to employ a parenteral route for therapeutic purposes.

This work has been supported by a grant from the Medical Research Council, which is gratefully acknowledged. We wish to thank Messrs. Vitamins, Ltd., for the preparation and gift of the vitamin solution.

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The Nuclear Membrane as a Barrier to the Free Diffusion of Proteins

A RECENT investigation of the osmotic properties of the nucleus in intact frog oocytes has indicated that the nuclear membrane of these cells is not freely permeable to large molecules (mol. wt. 40,000 or greater)[1]. The present investigation was carried out in order to determine whether or not similar results could be obtained for oocytes of an unrelated species.

Immature oocytes of the *Cecropia* moth (family, Saturniidae), in which yolk formation was just beginning and in which the nucleus was clearly visible, provided a suitable material for this purpose. The ovarioles were removed from animals that had completed one-third of the pupal-adult moult, and placed in a hanging drop of *Cecropia* pupal hæmolymph in a moist chamber. A 0·4 per cent solution of fluorescein-labelled rabbit γ-globulin (fraction II) in nine parts 0·151 *M* potassium chloride (in a sodium chloride solution which is isotonic with this concentration of potassium chloride, *Cecropia* oocytes will neither swell nor shrink: Telfer, W. H., personal communication) and one part 0·01 *M* phosphate buffer was then micro-injected into the cytoplasm of suitable oocytes. The *p*H of the above solution was 7·4; a value selected after injections of phenol red into the oocytes indicated a cytoplasmic *p*H of 7·0 or higher. The protein solution was injected near the nucleus, and care was taken to avoid contact between the micropipette and the nuclear membrane. No changes in the appearance of the cells were observed as a result of micro-injection. 10 min. after being injected, the oocytes were removed from the moist chamber and fixed for 20 min.–2 hr. in three parts 95 per cent alcohol and one part glacial acetic acid. After paraffin embedding, the cells were sectioned at 7 or 8μ. The paraffin was removed from the slides with xylene and the sections were mounted in Schillebar's oil (Grade *A*).

When the slides were observed with the fluorescence microscope, the fluorescence was found to be distributed throughout the cytoplasm. There was, however, no sign of fluorescence in the nucleus (Fig. 1). The results have been interpreted as meaning that the nuclear membrane of *Cecropia* oocyte acts as a barrier to the free diffusion of γ-globulin (mol. wt., approx. 165,000). The main criticism of this interpretation is that, following injection into the cytoplasm, the protein could have been removed from solution either by precipitation or by binding to certain cytoplasmic constituents. However, the fact that the fluorescence was widely distributed through the cytoplasm within 10 min. after the injection indicates that the mobility of the fluorescein-labelled

Fig. 1. A section through an injected oocyte showing the absence of fluorescein in the nucleus (*N*). Under the fluorescence microscope the nucleus appeared blue and the entire cytoplasm (*C*) was yellow. Due to a high concentration of granules, the fluorescence in area (*A*) did not show up with black-and-white photography

γ-globulin was sufficient for it to pass through the nuclear membrane if the latter were not impermeable.

It should be emphasized that the rather short 10-min. interval between injection and fixation was chosen for the specific purpose of determining whether the nuclear membrane can act as a barrier to the free diffusion of molecules having a large molecular weight. The inability of a protein to penetrate the nuclear membrane in short-term experiments does not necessarily rule out the possibility that proteins can actually pass into the nucleus. It is not inconceivable, as has been suggested by Stern and Mirsky[2], that an energy-requiring transport mechanism exists whereby molecules which cannot freely penetrate the nuclear membrane can enter the nucleus.

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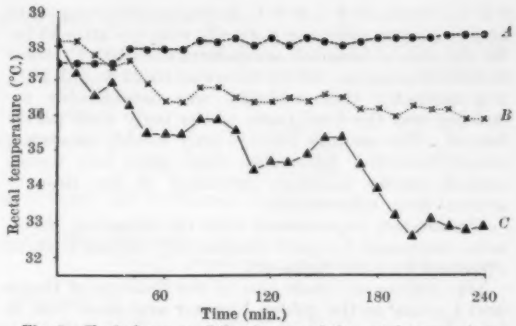

Fig. 1. Typical course of the changes of the rectal temperature in the European hamster in the experimental temperature − 8° C. (*A*), + 2° C. (*B*) and + 10° C. (*C*)

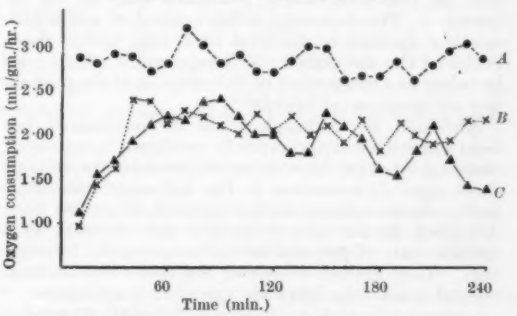

Fig. 2. Typical course of the changes of the oxygen consumption in the European hamster in the experimental temperature − 8° C. (*A*), + 2° C. (*B*) and + 10° C. (*C*)

Reaction of Hibernators to Various Low Temperatures of the Environment

IN order to establish the conditions in which hibernators do not maintain their constant body temperature and enter hibernation in summer, the following experiments were made with European hamsters (*Cricetus cricetus* L.) in July and September 1959. Animals were exposed to various low temperatures and their oxygen consumption and rectal temperature recorded for 4 hr. Examples of the results of these experiments are given in Figs. 1 and 2, and mean values of the oxygen consumption in Table 1.

It can be seen that in the experimental temperature − 8° C. the oxygen consumption of the hamsters rose very steeply at the beginning of the experiment to a high level which was maintained throughout the experimental period. Rectal temperature remained constant at the original value about 38° C. The animals behaved like true homoiotherms in these conditions.

In the experimental temperature + 10° C. the course of the changes in metabolism and rectal temperature was quite different. The initial rise of the oxygen consumption was slow and a maximum was not reached until the animals were cooled to some degree. Hereafter the oxygen consumption decreased slowly, but distinctly in a wave-like manner; in connexion with it the rectal temperature fell similarly, the decrease amounting in the mean to 5·2° C. within 4 hr.

In the experimental temperature + 2° C. reaction was intermediate between the two cases described: the oxygen consumption rose slowly, though more rapidly than in + 10° C., to a level which was lower than in − 8° C. and remained constant in the further course of the experiment. The rectal temperature fell rather rapidly during the first hour; later its fall was much slower.

The relative instability of the body temperature of hibernators even in periods of activity is well known[1-3]; it may, therefore, be questioned whether the fall of the rectal temperature observed in the experimental temperature + 10° C. after 4 hr. can be taken as evidence of a certain optimum temperature in which hibernation is induced. To obtain further evidence, animals were kept at a low temperature overnight (15 hr.) and their rectal temperature determined at the end of this period. The hamsters tolerated the temperature − 8° C. without any harm, their body temperature remained constant (38·2 ±

Table 1. MEAN VALUES OF OXYGEN CONSUMPTION IN THE EUROPEAN HAMSTER DURING EXPOSURE TO VARIOUS LOW TEMPERATURES OF THE ENVIRONMENT (SIX ANIMALS IN ONE GROUP)

Body-weight (gm.)	Experimental temperature (° C.)	Oxygen consumption ml./gm./hr. First hr.	Second hr.	Third hr.	Fourth hr.
295–315	− 8	2·92 ± 0·18	2·90 ± 0·22	2·81 ± 0·29	2·83 ± 0·21
285–315	+ 2	1·80 ± 0·25	2·12 ± 0·14	2·04 ± 0·11	2·03 ± 0·17
290–320	+ 10	1·75 ± 0·18	2·13 ± 0·25	1·83 ± 0·17	1·62 ± 0·09

0·3° C. before, 37·6 ± 0·6° C. at the end of the exposure in six animals) and were normally reactive after 15 hr. In the environmental temperature + 10° C. their rectal temperature fell in the mean to 22·6 ± 3·2° C. (six animals), their mobility was considerably restricted and the hind parts of the body were partly torpid. The animals reacted only weakly to strong sound stimuli; sometimes they gave out throat sounds similar to those produced at the time of arousal from hibernation.

Preliminary experiments with the hedgehog (*Erinaceus europaeus* L.) gave results very similar to those obtained with the hamster.

Our results are analogous to the findings of Deane and Lyman[4] in the golden hamster and show that in hibernators exposed to a high stress a thermoregulatory apparatus of an effectivity comparable to that of non-hibernating mammals can be set in function. The decreased reflex control of warm production[5] appears to manifest itself only within those limits of the environmental temperature which may be taken as a component of the complex of the permissive environmental factors[6].

Although the lethargic state in hibernators has been sometimes experimentally produced in summer[7], cases have not yet been described in which the animals show signs of transition to the lethargic state after such a short exposure to low temperature. The facts described do not as yet exclude the necessity of a specific state of preparation for inducing the hibernation. They favour, however, the view that environmental conditions have the greatest importance.

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Physiological Mechanisms concerned in the Maintenance of Lactation in the Goat and Sheep

THE anterior-pituitary galactopoietic hormones have been found to be essential for the maintenance of lactation in all species so far studied[1], and it is generally believed that the continued secretion of the galactopoietic complex is entirely dependent on nervous stimuli arising from the mammary gland[2].

Recently, some amplifications of Selye's theory have been put forward, and it has been suggested that the nerve stimuli may effect the release of prolactin and adrenocorticotrophic hormone from the anterior pituitary not directly but by way of the neurohypophysis through the intermediary of oxytocin[3] and perhaps vasopressin[4].

The experiments which gave rise to the neuroendocrine theory of the maintenance of milk secretion were carried out mainly on laboratory animals, and we thought it of interest to investigate whether the same conclusions are generally applicable to other species. For this purpose we have repeated many of these experiments on small ruminants (goats, sheep) and we have started by studying the effects of section of the afferent nerve pathways of the reflex. In a former experiment, the following procedure was carried out in goats and sheep during the first pregnancy.

Section of the lumbar nerves (first, second, third and fourth pairs), section of the perineal nerve with bilateral lumbar sympathectomy. In this experiment the operated animals, after parturition, gave considerable yields of milk.

Since this work was reported[5], we have carried out other experiments involving a greater degree of denervation of the mammary gland:

(1) Severance of all connexion between the mammary gland and the body except the inguinal blood vessels, with perivascular sympathectomy of inguinal blood vessels, followed by suturing of the gland back into position[6].

(2) Section of the spinal cord at *T*11 with bilateral sympathectomy and splanchnicectomy, also removal of the spinal cord between *T*11 and *L*5[7].

(3) Nerve section, resulting in insensitivity of the mammary gland and the adjacent areas, the experimental design and result of which are summarized in Tables 1 and 2. To eliminate any effects of possible regeneration of the perineal nerves, milk yields are given only for the first fifteen weeks of lactation although the yields were recorded over periods of 25–40 weeks. These results entirely confirm our previous experiments[5–7]. In a recent publication, Tverskoi[8] has similarly reported that cord section at *T*11 in the goat does not stop lactation.

Since, as the above findings show, goats and sheep can give substantial yields of milk in the absence of the milk-ejection reflex, it would seem that the milk-ejection reflex is not indispensable for lactation in these species.

The maintenance of the galactopoietic activity of the anterior pituitary in the absence both of the milk ejection reflex and of all mammary sensation may be explained in one of the two following ways. First, given currently accepted concepts of the maintenance of lactation, it may be supposed that some nerve fibres have remained intact, particularly in the deeper portions of the walls of the blood vessels. In this connexion the difficulty of achieving complete denervation is well known. This explanation, however, would only be valid if the fibres persisting in the walls of the inguinal vessels did not enter the main nerves supplying the mammary gland, but reached the spinal cord, at a level above *T*11 or the sympathetic chain above the thoracic ganglia through which the fibres of the splanchnic nerves pass. These assumptions, however, seem unlikely in view of anatomical studies of the nerve supply of the mammary gland[9]. Moreover, one would also have to assume that these hypothetical residual nerve fibres were concerned not with the transmission of stimuli inducing milk ejection or of painful tactile stimuli from the mammary gland, but were highly specialized and concerned entirely with the regulation of the secretory activity of the anterior pituitary. Our results make it difficult to believe that a small number of perivascular nerve fibres would suffice to transmit impulses sufficiently strong to regulate pituitary galactopoietic activity. Secondly, assuming that complete denervation of the udder has been achieved, it would appear to follow that secretion by the anterior pituitary of the galactopoietic complex is under humoral control.

Our experiments thus lead us to believe that the control of pituitary galactopoietic function may be

Table 1. Milk Yields of Ewes (First Lactation)

	Degree of denervation	No. of animals	Mean production per week for the first 15 weeks (ml.)	Total duration of lactation (weeks)
I*	Lumbar nerves 1, 2, 3, 4, lumbar sympathectomy, splanchnicectomy, perineal nerve, all bilateral section	6	4,083 ± 1,230	15·1
II*	Thoracic nerves 12, 13, lumbar nerves 1, 2, 3, 4, lumbar sympathectomy, perineal nerve, all bilateral section	3	4,537	21
III	Thoracic nerves 12, 13, lumbar nerves 1, 2, 3, 4, 5, lumbar sympathectomy, perineal nerve, all bilateral section	6	3,957 ± 997	20
IV	Total of operated animals	15	4,123 ± 1,583	18·7
V	Normal animals	21	4,481 ± 1,820	19·4

* In groups I and II, the lateral femoro-cutaneous nerve may still provide some innervation.

Table 2. Milk Yields of Goats (First Lactation)

	Degree of denervation	No. of animals	Mean production per week for the first 15 weeks (ml.)	Total duration of lactation (weeks)
I	Lumbar nerves 1, 2, 3, 4, lumbar sympathectomy, perineal nerve, all bilateral section	11	3,528 ± 1,385	18
II	Thoracic nerves 12, 13, lumbar nerves 1, 2, 3, 4, perineal nerve, all bilateral section	1	5,238	42
III	Thoracic nerves 12, 13, lumbar nerves 1, 2, 3, 4, 5, lumbar sympathectomy, perineal nerve, all bilateral section	3	2,300	46
IV	Thoracic nerves 12, 13, lumbar nerves 1, 2, 3, 4, 5, perineal nerve, all bilateral section	2	3,652	42
V	Lumbar nerves 1, 2, 3, 4, 5, perineal nerve, all bilateral section	2	3,270	23
VI	Total of bilaterally operated animals	19	3,418 ± 1,390	27
VII	Lumbar nerves 1, 2, 3, 4, lumbar sympathectomy, perineal nerve, all unilateral section	7	3,682 ± 1,610	26
VIII	Normal animals	13	6,753 ± 3,300	25

In addition to bilateral denervations, we performed a sympathectomy on inguinal vessels and a section of the spermatic nerve at the lower inguinal ring.

comparable to that envisaged by Fortier[10] in respect of the secretion of adrenocorticotrophic hormone. On this view two main types of stimuli would condition the maintenance of lactation in the normal animal, the relative importance of these two systems varying with the species. First, nervous stimuli would act on the anterior pituitary according to the concepts of Selye[2] or of Benson and Folley[3], the immediate reaction of the organism to the peripheral stimulation being discharge of hormone. Secondly, there may be systemic stimuli associated with the physiological mechanisms of general adaptation of the organism to the demands of the lactating mammary gland for water, metabolites and hormones. These stimuli might act either directly on the anterior pituitary or at the level of the hypothalamus.

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Urinary Œstrogens of the Dog

Investigations on naturally occurring œstrogens in the dog are few. So far as urinary œstrogens are concerned, controversial reports are given by authors using biological methods. Helm[1] and Küst[2] report negative findings for both non-pregnant and pregnant animals, while Lesbouyries and Berthelon[3], Finck[4], and Stiasny[5] found evidence for the presence of œstrogens in the urine of pregnant animals. Nilsson[6] reports positive values for non-pregnant bitches. In none of these investigations was the urine hydrolysed before assay.

So far as we know, no attempt has as yet been made towards characterization of the substances by chemical methods. In our laboratory the highly specific and sensitive method of Brown[7] for the chemical determination of œstrogens in human urine has been adopted for research in domestic animals[8]. This communication presents results indicating that by application of this method no significant amounts of œstrone, œstradiols and œstriol could be detected in the urine from male and from pregnant female dogs.

The material consisted of a batch of urine collected from three healthy male dogs, a batch from three healthy females during the last few days of gestation and a single specimen from another pregnant animal.

Two methods of hydrolysis were applied. In the recovery experiment on urines from males, hot acid hydrolysis was applied. A batch of 100 ml. urine was diluted to 200 ml. and boiled under reflux for 1 hr. using 15 vol. per cent hydrochloric acid. 20 μgm. quantities of free œstrogens were added to a parallel sample before acid hydrolysis. Pregnancy urine was submitted to enzyme hydrolysis, using a combined β-glucuronidase-phenolsulphatase preparation (1 ml. of the enzyme preparation contained 100,000 Fishman-units β-glucuronidase and 50,000–80,000 sulphatase-units). To 300 ml. urine was added hydrochloric acid to *p*H 5·0, and 60 ml. McIlvaine standard buffer (*p*H 5·0). After the addition of 1 ml. of the enzyme preparation and 100 mgm. of dihydrostreptomycin sulphate, the solution was incubated at 37° C. for 36 hr.

Quantitative determinations were then made on the different samples, using Brown's method[7] with slight modifications[8]. Values less than 1 μgm./l. of urine were considered insignificant.

Table 1. Recovery Experiment on Urine from Three Male Dogs

Œstrone		Œstradiol-17-β		Œstriol	
μgm. added	μgm. recovered	μgm. added	μgm. recovered	μgm. added	μgm. recovered
0	0	0	0	0	0
20	8·7	20	7·9	20	11·8

The œstrogens were added *before* acid hydrolysis.

Table 2. Œstrogens in the Urine of Pregnant Bitches

Method of hydrolysis	Urine specimen	Œstrone	Œstradiol-17-β	Œstriol
Enzymic	Pooled from three bitches in late pregnancy	0	0	2·2
1 hr. reflux with 15 vol. per cent hydrochloric acid	Fresh from a single pregnant bitch	4·0	0	13·0
	Same urine after storage for 18 months at 4° C.	0	0	0

All values are uncorrected and represent μgm. per litre urine.

Results for male urine in Table 1 indicate that it is practically free from the œstrogens searched for. The recoveries lie well within the limits found by Diczfalusy and Westman[9] for human urine using a similar method.

As shown in Table 2, urine from pregnant bitches appears to contain only trace amounts of the œstrogens searched for. As enzyme hydrolysis, in our opinion, is considerably more efficient than acid hydrolysis, and is not known to cause destruction of liberated œstrogens, these observations can be considered valid. The presence of œstrogens other than those covered by the method applied in the present investigation can, of course, not be excluded. But generally, there seems to be agreement that the œstrogen-level in the urine of the dog is very low, regardless of physiological state. Our results tend to confirm this view.

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PLANT PHYSIOLOGY

A Possible Relationship between Nucleic Acid Metabolism and the Initiation of Zygospores of *Rhizopus sexualis*

It has been observed[1] in the homothallic fungus *Rhizopus sexualis* (Smith) Callen that the initiation and early stages of maturation of zygospores, which at 20–25° C. occur within a few days of inoculation, are completely inhibited at temperatures below 10° C. Later stages in the maturation of zygospores initiated at room temperature and afterwards transferred to low temperature are not inhibited; this suggests that a possible cause of the inhibition is a block in the synthesis of some compound or compounds essential for the early stages of zygospore production.

Some evidence is now available that the first stages of zygospore development are associated with a change in the nucleic acid metabolism of the organism. The nucleic acid content of cultures at different stages of development has been investigated by a procedure based on the perchloric acid extraction method of Ogur and Rosen[2], and it has been found that the level of total nucleic acids (either per culture or per unit dry weight of mycelium) reaches a maximum at the time of zygospore initiation and then falls. By a technique based on that of Korson[3] and used for fungi by Griffiths[4], the ribonucleic acid and the deoxyribonucleic acid of the cells can be differentially stained. This reveals that a rather high concentration of ribonucleic acid is maintained in the hyphal tips, up to the time of zygophore differentiation. Afterwards the level of ribonucleic acid falls rapidly; this fall appears to be accompanied by a rise in the deoxyribonucleic acid content, but later observations are made difficult by the pigmentation of the zygospore wall. In cultures in which zygospore formation is inhibited by low temperature the level of ribonucleic acid remains high. These results suggest that the deoxyribonucleic acid synthesis accompanying zygospore development may be at the expense of the ribonucleic acid of the mycelium, and that the inhibition noted at low temperature is due to a blockage of synthesis of deoxyribonucleic acid. A similar effect of low temperature upon the transformation of ribo- to deoxyribo-nucleic acid in higher plants has long been known[5].

Investigations of the effect of mature zygospores upon further zygospore initiation and development in the same culture resulted in the observation that a volatile compound is produced by mature cultures and is capable of stimulating zygospore production in younger cultures kept at room temperature and, in some instances, of initiating zygospore formation at low temperature. Mature cultures of related species of fungi also produce volatile substances with similar, although smaller, effects on cultures of *R. sexualis*. The activity is not due to carbon dioxide or ammonia, or to a lowering of oxygen tension. The active compound is apparently basic, since it can be removed from a gas stream by scrubbing with dilute hydrochloric acid and is not removed by potassium hydroxide. Attempts to identify the compound by means of a mass spectrometer have been inconclusive, but the fairly frequent occurrence of peaks of mass 15 and 16 is consistent with the possibility that the compound is methylamine. So far as is known, methylamine has not been reported as a metabolic product of *R. sexualis*, but it would not be surprising if it were formed in small amounts, especially in older cultures. When methylamine was supplied at the rate of 1 μgm. per hr. in the air stream passing over cultures at 9° C., there was evidence of a partial reversal of inhibition, in that irregular hyphal swellings similar to pseudophores were induced. In cultures in which zygospore initials were present before chilling, methylamine increased their rate of maturation. Other compounds, such as serine, choline and, to a lesser extent, formic acid, were effective when supplied with the culture medium in increasing zygospore production in cultures in which initiation had already begun. Methionine, betaine and glycine had no effect.

The evidence at present available suggests that the effect of low temperature on zygospore formation is to inhibit the interconversion of ribo- and deoxyribonucleic acid; this inhibition is at least partially reversed by chemicals which are closely associated with the metabolism of methyl and other monocarbon compounds in the cell[6]. In this connexion

it is of interest to note that a major difference between ribo- and deoxyribo-nucleic acids lies in the presence of the pyrimidine uracil in ribonucleic acid and its replacement by thymine (5-methyl uracil) in deoxyribonucleic acid. Some of the chief metabolic sources of the methyl moiety of thymine incorporated in the deoxyribonucleic acid of young rats have been found to be serine and choline[7]. It is tempting to speculate that the non-availability of methylating agents needed for the synthesis of thymine and deoxyribonucleic acid may be the chief factor in the low-temperature inhibition of zygospore formation in *R. sexualis*. Critical experiments to test this hypothesis are in preparation, and it is hoped to publish a fuller account of this work when further results are available.

We wish to thank Dr. L. E. Hawker for introducing us to this problem, and for her help, interest and encouragement throughout the work. We are indebted to the Department of Scientific and Industrial Research for a studentship for one of us (P. M. H.).

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Effect of Gibberellic Acid on Hypocotyl Growth of Lettuce Seedlings

Gibberellins are physiologically defined by their ability to induce shoot elongation in certain dwarf plants and they have been demonstrated in various plant extracts using dwarf maize plants[1] and dwarf peas[2–4] as assay material. Other assay material includes leaf sections of wheat seedlings[3] and sections of cucumber tendrils[5]. A convenient method employed by Murakami[6] involves the use of rice seedlings. Dwarf maize and pea plants, although very sensitive, are less convenient for assaying the several fractions obtained after paper chromatographic separation of plant extracts. An attempt was made to develop a simple means of bio-assay for this purpose.

The internode elongation shown by dark-grown pea seedlings can be reproduced in light-grown seedlings by gibberellic acid treatment. This has, in fact, been used as a bio-assay technique[7]. In seeds with an epigeal type of germination there is elongation of hypocotyl tissue as well as of internodes in the absence of light and it was decided to investigate the effect of gibberellic acid on hypocotyl growth in three species: *Lactuca sativa* (variety Grand Rapid), *Lepidium sativum*, *Hyoscyamus niger*. There have recently been brief reports[8,9] of the stimulatory effect of gibberellic acid on lettuce seedling growth.

Lettuce seeds were sown in the dark at about 25° C. After 2 days seedlings were selected with radicles of a similar length (6–8 mm.) and planted in 4 in. Petri dishes lined with filter paper moistened with 6 ml. of test solution. 10 seedlings were used per treatment. They were placed 15 cm. below a light source consisting of two daylight fluorescent tubes (5 ft., 80 watt), in which position the temperature was about 28° C. Hypocotyl length was recorded after 5 days, each hypocotyl being measured to the nearest mm. Gibberellic acid had a significant effect on hypocotyl growth at concentrations of 0·1 mgm./l. and above. Growth in 100 mgm./l. gibberellic acid was almost as great as growth of untreated seedlings in the dark. There was a linear relationship between hypocotyl elongation and the logarithm of gibberellic acid concentration. In the dark, hypocotyl growth was affected very little by gibberellic acid treatment. In subsequent experiments measurements were taken after 3 days and seeds were germinated in continuous light. This increased the light inhibition of hypocotyl growth but did not increase sensitivity to gibberellic acid.

Table 1. Effect of Gibberellic Acid on Hypocotyl Extension of Lettuce Seedlings

Treatment, gibberellic acid in mgm./l.	Mean hypocotyl length ± *S.E.*	
	A	*B*
Untreated	6·0 ± 0·21	4·2 ± 0·13
0·01	6·0 ± 0·21	4·7 ± 0·21
0·1	9·7 ± 0·45	7·3 ± 0·33
1	13·1 ± 0·43	11·6 ± 0·54
10	17·3 ± 0·36	13·5 ± 0·40
100	19·1 ± 0·55	—
Untreated (dark)	20·3 ± 0·71	—
100(dark)	21·8 ± 0·74	—

A, Lettuce seedlings germinated in dark prior to treatment. Measurements taken after 5 days. *B*, Lettuce seedlings germinated in light. Measurements taken after 3 days.

To investigate the specificity of the growth-response to gibberellins light-grown lettuce seedlings were also treated with the auxins, β-indoleacetic acid and 2-naphthoxyacetic acid. In neither case was there any positive growth response. Also sucrose had no effect on hypocotyl growth.

Hypocotyls of *Lepidium* and *Hyoscyamus* also showed positive growth responses to gibberellic acid but the effect was less marked than in lettuce.

The following procedure was employed when the lettuce hypocotyl test was used for assaying plant extracts following paper chromatographic separation. The extract was strip-loaded on a 10 cm. wide strip of Whatman No. 1 chromatography paper and developed in a descending fashion with a suitable solvent. The developed chromatogram was cut into ten transverse strips, each strip being cut into two and placed in a Petri dish on filter paper moistened with 6 ml. of water. Two-day-old lettuce seedlings were then planted on the chromatogram strips and hypocotyl growth measured after 3 days.

Using this technique it was possible to detect gibberellin-like activity in plant extracts, although the success of the bio-assay was mitigated by the presence of inhibitory substances in the extract, preventing true quantitative estimates from being made. It is intended to try out further chromatographic techniques which will effect more efficient separation of gibberellins from inhibitory substances.

The chief merit of this lettuce hypocotyl test lies in its simplicity. Seedlings are available for the bio-assay within 48 hr. of sowing and are small enough to be grown directly on the solution under test.

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IMMUNOLOGY

Inhibition of the Inductive Phase of Antibody Formation by 6-Mercaptopurine examined by the Transfer of Isolated Cells

The inductive phase of antibody formation is a period that differs not only quantitatively but also qualitatively from the later productive phase[1]. This is seen from the effects of X-irradiation[2] and of cortisone[3] on antibody formation, and in particular from the results of tissue-culture experiments[4].

When inhibitors act on the whole organism, however, it is impossible to determine reliably whether they affect the inductive phase only, or also the productive phase. Antigen persists in the tissues for at least a week and during this time the mesenchymal stem cells normally proliferate. This gives rise to a situation in which the newly developing cells are stimulated by antigen (commencement of the inductive phase), while cells which were previously in contact with the antigen are in the productive phase. Since the outcome of the effect of inhibitors on the organism is not instantaneous, it is not always easy to decide during which phase the cells are affected. I therefore examined the effect of inhibitors on a suspension of isolated spleen cells transferred to newly born rabbits[5].

One of the inhibitors selected was 6-mercaptopurine, in an attempt to verify the working hypothesis that ribonucleoprotein, which is responsible for the synthesis of specific protein (antibody) during the productive phase, is itself synthesized during the inductive phase[6]. In earlier experiments 6-mercaptopurine had been used to inhibit antibody formation in adult rabbits immunized with *Salmonella paratyphi B* antigen[7]. Using *S. paratyphi B*, antibody formation was not inhibited even by toxic doses of 6-mercaptopurine, evidently because this antigen evokes a secondary reaction. When Schwartz, Stack and Dameshek[8], however, later used protein antigen ('primary reaction'), they obtained complete inhibition of antibody formation by 6-mercaptopurine.

In the present experiments, 1 ml. spleen cells isolated by the method described in a previous paper[5] was administered intraperitoneally to young rabbits; 1 ml. contained 50×10^6 cells. In all the experiments 6-mercaptopurine was administered subcutaneously in amounts of 0·5 mgm./100 gm. body-weight daily.

In the first experiments, cells from normal unimmunized donors, mixed *in vitro* with heat-inactivated

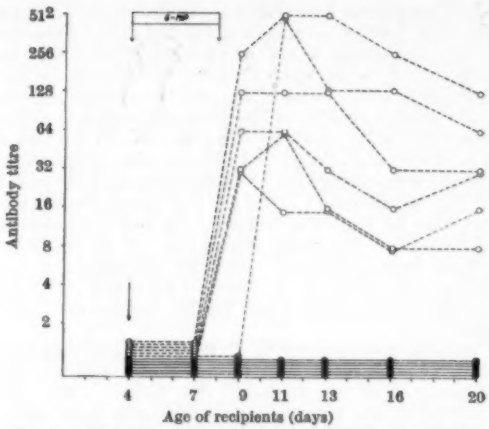

Fig. 1. 6-Mercaptopurine administered simultaneously with transfer of normal cells mixed with *B. suis* antigen *in vitro* (full line). The same cell suspension was administered without 6-mercaptopurine to control recipients (interrupted line)

Brucella suis antigen (7×10^8 micro-organism), were administered. In animals which received 6-mercaptopurine on the first five days after cell transfer, antibody formation was completely inhibited. Cells administered without the simultaneous administration of 6-mercaptopurine formed antibodies in the normal manner (Fig. 1).

In other experiments, cells from an immunized donor, that is, already forming antibodies, were transferred. The administration of 6-mercaptopurine in the same doses and at the same times did not prevent these cells from forming antibodies (Fig. 2). These results demonstrate that 6-mercaptopurine does not have an inhibitory effect if antibody formation has already developed (that is, during the productive phase).

In order to determine more precisely the phase of antibody formation as affected by 6-mercaptopurine, this substance was administered at different time-intervals after transfer of normal cells mixed with antigen *in vitro*. When administered 24 hr. after cell transfer, it inhibited antibody formation in every

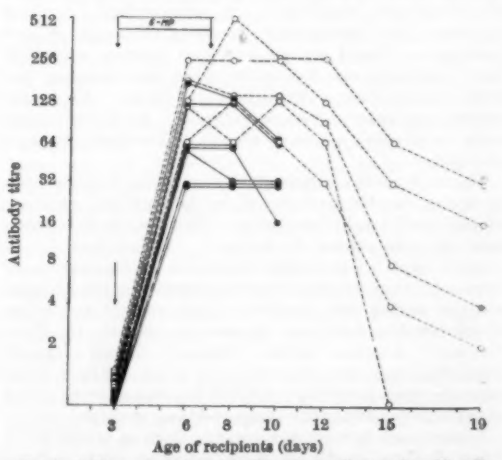

Fig. 2. 6-Mercaptopurine administered simultaneously with cells from previously immunized donor (full line). The same cell suspension was administered, without 6-mercaptopurine, to the controls (interrupted line)

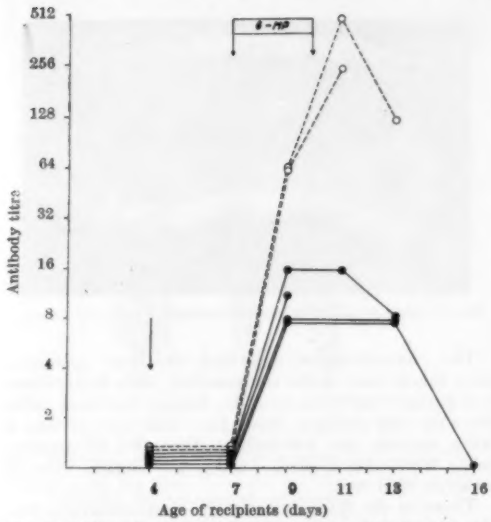

Fig. 3. 6-Mercaptopurine administered 72 hr. after transfer of normal cells incubated with *B. suis* antigen *in vitro* (full line). The same cell suspension was administered, without 6-mercaptopurine, to the controls

case. If it was administered 72 hr. after transfer, the transferred cells formed antibodies, although in low titres only (Fig. 3). Using a bacterial antigen, therefore, the crucial period for sensitivity to 6-mercaptopurine is 48–72 hr. after injection of the cells mixed with antigen *in vitro*; this corresponds exactly to the inductive period, as determined in tissue cultures[4].

It was usually found during these experiments that the dose of 0·5 mgm. 6-mercaptopurine/100 gm., which normally is tolerated well, was lethal for young animals to which cells already forming antibodies were transferred (Fig. 2). This is probably due to summation of the effect of 6-mercaptopurine and the metabolic demands of the transferred antibody-producing cells.

The results show that 6-mercaptopurine is effective during the inductive phase only; the mechanism of its inhibitory action supports the conception that processes during the inductive phase are associated with the synthesis of nucleoprotein. These results form part of a wider series of transfer experiments in which different types of inhibitors (for example, colchicine, ethionine) are used in an attempt to define exactly the conditions and metabolic processes of the inductive phase of antibody formation.

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BIOLOGY

Myxomatosis in Britain

In his article, Dr. C. H. Andrewes[1] directs attention to the contrast between the host/vector relationship in Britain and Australia. When we have acquired further information about the biology of the rabbit flea (*Spilopsyllus cuniculi*) and also other possible arthropod vectors, we may find that a somewhat different set of factors comes into operation in Britain between major epizootics.

After the initial outbreak of the virulent type of myxomatosis, when the rabbit population at Ashton, Peterborough, was at its lowest ebb, the few survivors which were caught showed a greatly reduced individual flea infestation. It might have been supposed that, owing to the number of dead rabbits from which the fleas had scattered in search of living hosts, the remaining healthy rabbits, especially any immune survivors, would become progressively more heavily infested. But this was not so. All survivors examined six months after the first great epizootic harboured at the most four or five fleas each, quite frequently no fleas at all, or fleas other than rabbit fleas. In the year preceding the outbreak 500 rabbits examined from the same area showed a 100 per cent infestation-rate with a mean per rabbit of approximately 70 fleas on the small sample counted. Three years later, when rabbits were becoming more numerous at Ashton, rabbit fleas were again plentiful, although no actual counts were made at this time.

It therefore seems possible that the chief vectors during a big epidemic may be different from those between, or right at the end, of a big epizootic. Mosquitoes spread myxomatosis, at any rate to a limited extent, in Britain[1]; and it is probable that other winged vectors such as black fly (*Simulium*) are capable of transmitting the disease. During the period of reduced rabbit and flea population the winged vectors may well play the major part as agents of dissemination, and if this were so, the avirulent strains of myxomatosis would, between big epizootics, enjoy certain advantages similar to those described for these strains in Australia. When the rabbit and flea populations build up again, which they do fairly rapidly, the rabbit flea would assume once more its role as chief vector in Britain and consequently there would again be some evolutionary pressure in favour of a virulent virus.

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Glucose-6-Phosphate Dehydrogenase Deficiency Trait in Nigeria

We were led to look for the presence of this trait in Nigeria because of the demonstration that 10 per cent of American Negroes are deficient in this enzyme[1] and the discovery that hæmolytic anæmia caused by certain drugs (for example, primaquine) develops only in the presence of this sex-linked inherited deficiency of red cell glucose-6-phosphate dehydrogenase[2].

We have applied the rapid screening test as described by Motulsky[3] to 204 male Nigerians of

which 139 were Ibo subjects, all of them inmates of Uzuakoli Leprosarium, and 65 were Yoruba subjects attending University College Hospital, Ibadan. Assay for glucose-6-phosphate dehydrogenase[3] was carried out on all 20 persons found deficient by the rapid screening test, and on 18 normal subjects. Our results are shown in Table 1.

Table 1. Incidence of Deficiency of Glucose-6-phosphate Dehydrogenase in Red Cells of 200 Male Nigerians

Tribe	No. examined	No. deficient
Ibo	139	9
Yoruba	65	11

The demonstration of the presence of the trait in Nigeria raises several interesting problems: (1) The suggestion made by Motulsky[3] that subjects lacking the enzyme may exhibit a relative resistance to falciparum malaria could be investigated by field-studies of children less than five years of age, and by a study of severely ill malarial children admitted to hospital. (2) The presence of the trait may determine the onset of hæmolytic anæmia induced by dapsone in Nigerian leprosy patients, the incidence of which is approximately 10 per cent (Davey, F., personal communication). (3) The tribal distribution of the trait will have to be assessed in a far larger group of unselected persons. (4) The presence of the trait in adult Nigerians should be looked for in cases of unexplained hæmolytic anæmia often summarily dismissed as 'blackwater fever'.

The co-operation of Drs. F. Davey and L. Hogerzeil in obtaining blood samples is greatly appreciated.

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A New Record of *Mytilicola intestinalis* Steuer, a Parasitic Copepod of Mussels

Since the description of *Mytilicola intestinalis* by Steuer in 1902[1] from Trieste, it has been found in various localities of the Mediterranean and appears to have spread from this area to the Atlantic seaboard of Europe and around the British Isles[2]. Infection with this parasite has resulted in serious damage to mussel beds in many areas, with an attendant effect on the mussel industry[3]. Any information regarding the spread of this parasite is consequently of some importance. It is therefore interesting to record the taking of two female *M. intestinalis* in the Indian Ocean–Malacca Strait area, position 7° 12′ N., 97° 12′ E.

The two specimens taken (Fig. 1), approximately 8·5 mm. and 7·0 mm. long, were sufficiently distinctive to refer to this species rather than to either of the two other described species, namely, *M. orientalis* Mori and *M. porrecta* Humes. Dr. Krishnaswamy, of the University of Southampton, has kindly examined the specimens for me and agrees with the identification.

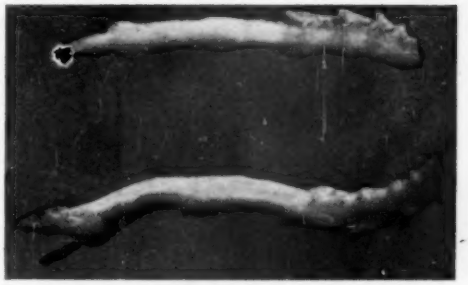

Fig. 1. Adult female *Mytilicola intestinalis*, 8·5 mm. and 7·0 mm. long

The circumstances in which the two specimens were taken were quite remarkable, both being taken in a surface plankton sample, during darkness, while the ship was drifting, hove to. The station was 50 miles outside the 100-fathom line and 67 nautical miles from the nearest land; the depth was 520 fathoms (951 m.).

There is no question of any contamination from European sources, nor is there the possibility that they had been carried from the shore since the water was clear blue and typically oceanic, as was the plankton. The possibility that they had been carried to the surface by currents is considered non-existent. Both my colleague on the cruise and myself are of the opinion that there was no growth of mussels on the ship's hull, although the possibility cannot be ruled out entirely (*M. viridis* L. is the common *Mytilus* of this area). Even if there were infected mussels on the hull it is difficult to explain how the parasites left the host and entered the net, which was some yards from the hull. The possibility that they had been regurgitated by a mussel-eating fish or bird is discounted in view of the excellent condition of the specimens. That adult female *Mytilicola* can be planktonic appears doubtful since the parasite has so far been taken only inside the mussels and never free.

However this may be, the occurrence of this species in the Malacca Strait extends its range of distribution, and increased collection in other areas may well prove the species to be more widely distributed than was hitherto known.

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First Successful Hybrid between the Two Jute-yielding Species, *Corchorus olitorius* L. (Tossa) × *C. capsularis* L. (White)

Although numerous attempts had been made in the past to cross the two jute-yielding species, namely, *C. olitorius* and *C. capsularis*, no success was achieved[1]. The recent report by Ganesan *et al.*[2] that following hybridization between the two species a few-celled embryo and few free endosperm nuclei are formed encouraged us to repeat the above crosses. The failure to obtain hybrid seedlings has been attributed by these authors to the degeneration of embryo and endosperm at an early stage of their development. An attempt was therefore made to induce the full

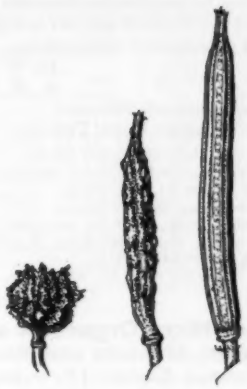

Fig. 1. Fruit shape and size in the hybrid and parents. *a*, Spherical fruit of *C. capsularis*; *b*, hybrid, note the projections and depressions along the ribs; *c*, 10-ribbed elongated fruit of *C. olitorius*, ribs are parallel and smooth. × ½

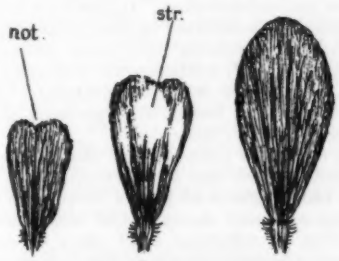

Fig. 2. Petal shape and size in the hybrid and parents. *a*, Notched petal of *C. capsularis*; *b*, notched petal of hybrid with irregular white streaks running from apex to base; *c*, petal of *C. olitorius*. *not.*, notch; *str.*, streak. × 5

development of the embryo and endosperm by the use of hormones. Following pollination, therefore, the pedicels of the flowers were wrapped with cotton which had been previously soaked in 300 p.p.m. 3-indole acetic acid. It was observed that the application of hormone increased the fruit set in the crosses and the seeds obtained therein appeared more developed than in fruits without hormone treatment. Of the 365 seeds obtained from 15 fruits in 115 crosses between *C. olitorius* (♀) and *C. capsularis* (♂), only 7 germinated, and of these only 3 grew to adult plants. No hybrid was obtained in the reciprocal cross.

All the 3 hybrids were slow growing, smaller in height and had a smaller base diameter compared with the parents. The hybrids showed dominance of the female parent in respect of the presence of a pair of stipules, shape of buds and fruits. In the character of leaf shape, B/L ratio of leaf, petal shape, presence of a notch on the upper margin of the majority of petals, irregularly ribbed fruits with alternate projections and depressions running along the ribs, the hybrids showed dominance of the *capsularis* parent (Fig. 1). The hybrids were intermediate in respect of serration of leaf margins, shape of bud and flower, colour and size of petals (Fig. 2), number of stamens and pollen diameter. In addition, the leaves of the hybrids showed areas of yellowish-green colour surrounded by normal green pigment. The petals showed irregular white streaks (Fig. 2*b*) running from the apex to the base surrounded by normal yellow pigment. The presence of these probably indicates the interference in the production of pigmentation brought about by new genic combination. Such disturbance in the production of pigmentation has been reported previously by Harland[3] in the progeny of interspecific crosses of *Gossypium* species, for example, *hirsutum–barbadense* crosses. The first few flowers in the hybrids withered away. Even selfing did not help in the setting of fruits. Almost 50 per cent of the flowers afterwards formed developed into fruits. These fruits varied a great deal in their size and the number of good seeds they contained.

All the 55 back-crosses to the *capsularis* parent failed. One hundred and twenty back-crosses to *olitorius* resulted only in the formation of 7 fruits.

All the diakinetic nuclei studied in the hybrids showed 7 bivalents. One of the 7 bivalents showed incomplete pairing. The two chromosomes in this pair were of unequal length. In all the diakinetic nuclei studied only one pair of chromosomes was found to be attached to the nucleolus. Except for the presence of two univalents in a small number of pollen mother cells, mostly 7 bivalents were seen at metaphase I. Lagging chromosomes at M_I and M_{II} were seen in only a small percentage of pollen mother cells. These irregularities were reflected in the presence of nearly 25 per cent sterile pollen grains.

It is known that cryptic structural hybridity obscures the true homology of the parental chromosomes of the hybrid as differences in the small segments of chromosomes do not interfere in the pairing of homologous chromosomes. Whether chromosome pairing in the hybrid was of this type or it really expressed the close homology between the chromosomes of the two species could not be ascertained due to the smallness of chromosomes.

The factor which led to the degeneration of embryo and endosperm at an early stage may have been counteracted by 3-indole acetic acid. The inhibitory effect being removed, the embryo and endosperm developed fully.

The climatic condition and weather in Hyderabad are different from those obtaining in Bengal and this may have also partly contributed to the success of the cross.

The hybrids have opened up new possibilities to jute breeders because by means of selection through a number of generations it may be possible to obtain a pure line with the desirable qualities of both the species combined.

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Presence of *Aphelenchoides cocophilus* in the Roots of *Cocos nucifera*, the Coconut Palm

Literature on red ring disease of coconuts, caused by the nematode, *Aphelenchoides cocophilus*[1,2], leaves the reader in doubt regarding the primary site of infection. Nowell[2] referred to the disease in Grenada as 'root disease' and considered the roots to be the

primary site of infection. Later[4], he changed his opinion and regarded root infections as secondary to stem. Other workers[5,6] suggested that the disease was transmitted by the palm weevil, *Rhyncophorus palmarum* (L.). One of us[7] advanced the view that the disease frequently started in the base of the tree and suggested that infection might occur via the roots.

While making a series of observations on the disease in the field, we detected the presence of *Aphelenchoides cocophilus* in the roots of three apparently healthy coconut trees. As a result of this, we conducted a survey of a young plantation and examined the roots of 171 trees; root infections were detected in 55 of these trees; two of these root-infected trees were suffering from red ring infection of the stem.

The distribution of the worm was investigated in a number of individual trees and the maximum density was usually found 4–8 ft. away from the bole, and a continuous distribution of nematodes was detected for a distance of up to 14 ft. from the bole. The worms were usually found in the lateral surface roots down to a maximum depth of about 12 in. below the surface; the density of worms in these roots never approached that found in the stem tissues and rarely exceeded 200–300 worms per gm.

Trees, the roots of which were infected, frequently showed symptoms which we considered as indicative of root damage. The most outstanding symptom was a drooping of the bunches of nuts when these were present and a wilting and drooping of the lower leaves: there was no evidence of yellowing. Sometimes when roots of trees showing these symptoms were examined, *Aphelenchoides cocophilus* was not found, but many living specimens of *Rotylenchus* sp. were recovered.

Roots infected with *Aphelenchoides cocophilus* differ from healthy roots in that the root cortex which is firm in texture and white in colour in healthy roots assumes an orange-red coloration and becomes soft and spongy. These symptoms in the roots are not specific to *Aphelenchoides cocophilus* nor, for that matter, to nematode infection, but can also be induced by a variety of other causes.

Preliminary observations suggest that initial infection of roots occurs some distance from the bole, and spreads inwards; but further work is necessary to establish this. The presence of worms in the distal parts of the root system of apparently non-infected trees, when these roots mingle with the infected roots of adjacent trees, suggests the transmission of worms from tree to tree via the root system.

The plantation in question has been observed for four months since root infections were first detected. During this time 7 of the 55 root-infested trees have succumbed to red ring disease; in six of these, the distribution of red ring was consistent with infection starting at the base; the other was definitely the result of an infection starting in the crown. Of the 116 trees in which no root infection was detected only one tree has succumbed; in this, although the characteristic red ring only extended up a distance of 4–5 ft. and there were no petiole infections, yet worms were recovered from the root system for a distance of up to 10 ft. from the bole at one side. The majority of the root-infested trees which succumbed to the disease did so two or three months after root infection was first detected, so it is probable that *Aphelenchoides cocophilus* can remain in the root system for a considerable time before infecting the stem.

It is necessary to emphasize that the observations herein recorded are limited and do not as yet permit the drawing of generalized conclusions.

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Effect of Micro-Organisms on the Development of Roots and Root Hairs of Subterranean Clover (*T. subterraneum*)

Although considerable information is available on the rhizosphere microflora and the influence of plants on this population, relatively little is known of the effects of these micro-organisms on the host plant apart from organisms such as *Rhizobium* spp., plant pathogens and mycorrhizas. It has been shown that root growth can be affected by the filtrates of cultures of rhizosphere organisms[1] and some antibiotic substances (polymyxin[2], streptomycin and griseofulvin[3]); however, in all these cases the products of microbial growth rather than the micro-organisms themselves have been added to the roots.

In the course of some general rhizosphere studies being conducted in this laboratory, it was observed that the root systems of plants inoculated with soil suspension appeared stunted and were more easily washed free of adhering sand than corresponding sterile root systems. Some of the results of subsequent studies are reported in this communication.

Seed of subterranean clover was surface sterilized with calcium hypochlorite, germinated on agar and non-contaminated seeds transferred to tubes of autoclaved sand and agar deeps. The sand was in 3 cm. × 20 cm. tubes to a depth of 5 cm. and brought to the equivalent of field capacity with plant nutrient solution[4]; three plants being grown in each tube. The agar deeps were in 1·5 cm. × 25 cm. tubes with 15 cm. of 1 per cent agar prepared with plant nutrient solution; one plant being grown in each agar deep. One half of the tubes were inoculated with 0·1 ml. of a 1-in-10 suspension of pasture soil, the other half receiving 0·1 ml. of a similar soil suspension which had been autoclaved. The tubes were transferred to a controlled-environment room which provided a 12-hr. light period each day of 1,000 ft.-candles on the plants with day and night temperatures of 18° C. and 15° C. respectively. The daily increments in growth of roots in agar were measured, while plants growing in sand tubes were harvested twice each week for root-length measurements and root-hair observations. Twelve replicates of each treatment were set up in the agar series and 12 plants harvested from each of the sterile and non-sterile sand treatments for each observation.

The mean daily root-length increments for sterile and non-sterile clover plants grown in agar (Table 1) show that elongation of the tap root was considerably reduced in the presence of micro-organisms, although there were no indications of root invasion or damage due to pathogens. The standard deviation for the daily measurements shown in Table 1 indicates a

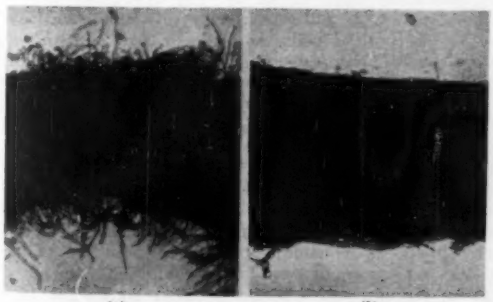
Table 1. Mean Daily Increments in the Growth of Sterile and Non-sterile Subterranean Clover Roots in Agar

Days after planting	Daily increment (mm.) Sterile	Non-sterile	Days after planting	Daily increment (mm.) Sterile	Non-sterile
1	4·0 (1·2)*	4·2 (1·6)	12	4·2 (0·66)	1·9 (1·5)
2	3·7 (1·2)	3·0 (0·89)	13	4·4 (0·42)	1·8 (1·1)
3	3·9 (1·4)	2·2 (2·5)	14	5·4 (0·70)	2·0 (1·1)
4	6·0 (1·2)	2·8 (2·2)	15	5·0 (0·72)	1·2 (1·2)
5	7·5 (2·2)	4·9 (2·4)	16	5·5 (0·90)	2·1 (1·1)
6	7·9 (2·5)	5·0 (3·2)	17	4·8 (0·81)	1·9 (1·3)
7	7·1 (1·3)	6·4 (3·7)	18	4·8 (0·63)	1·8 (1·2)
8	6·2 (1·1)	4·7 (3·3)	19	5·0 (0·77)	1·6 (1·4)
9	6·2 (1·6)	4·6 (3·5)	20	5·1 (1·0)	1·6 (1·2)
10	4·0 (0·98)	2·7 (2·2)	21	5·3 (1·1)	1·6 (1·1)
11	5·0 (0·89)	3·6 (3·2)	22	4·8 (0·78)	1·2 (1·1)

* Standard deviation.

much greater variability among the non-sterile plants which may be due to different types and numbers of micro-organisms on the different roots. The stunting effect of micro-organisms was also observed to a lesser degree in plants growing in sand. The growth of the plant tops did not seem to be affected by the micro-organisms on the roots, but this may not be so where the plant nutrients are not in luxury amounts as is the case in Hoagland and Arnon's nutrient solution.

Observations of the root hairs of plants grown in agar and sand showed that these were fewer in number and shorter in length in the non-sterile treatments. Fig. 1 shows the root hairs on the mid-root portions of sterile and non-sterile clover root systems grown in sand for six weeks. The effect was even more marked in the lower root portions—especially near the tip where the non-sterile were completely devoid of root hairs whereas the sterile roots supported large numbers of root hairs.



(a) (b)

Fig. 1. Root hair development on the mid-root portions of (a) sterile and (b) non-sterile subterranean clover roots in sand

Similar effects of micro-organisms on root development have also been recorded for tomato, *Phalaris tuberosa* and *Pinus radiata* growing in sand and agar, but the degree of stunting of roots and root hairs varied according to the plant and environmental conditions. This suggests a complex interaction between plants and micro-organisms. A more detailed report of the findings is to be published elsewhere.

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RADIOBIOLOGY

Conditioned Avoidance Behaviour induced by Low-Dose Neutron Exposure

Previous studies have demonstrated that exposure to low-intensity γ- or X-rays over a period of 1 hr. or more leads to aversive behaviour in rats[1–4]. The avoidance behaviour is a learned response dependent upon an association between discriminative stimuli and exposure to radiation. The most sensitive test developed so far involves the use of distinctive taste stimuli. Rats will normally consume saccharin-flavoured water in preference to tap water. However, if a rat tastes this solution during a 6-hr. exposure to gamma-rays, resulting in a total dose of 30 r., it will exhibit a marked decrement in preference for saccharin which persists for several weeks post-irradiation. In contrast, an animal which drinks tap water during a similar exposure will maintain the normal preference for the saccharin solution.

The 60-in. cyclotron on the Berkeley California campus of the University of California was employed in this study to determine if fast neutron bombardment would establish avoidance conditioning in rats. The present work differs from previous studies in several important variables of radiation exposure. The animals were exposed for a brief period to fast neutrons at a relatively high dose-rate whereas in previous studies the animals were exposed for several hours to γ- or X-ray radiation at very much lower dose-rates.

Young male rats, approximately 90 days of age, of a Sprague–Dawley strain bred at this laboratory, were used in this study. Litter-mate animals were distributed into control and experimental groups in a balanced design. On three occasions during the two weeks prior to neutron exposure, the animals were processed through sham-irradiation procedures for the purpose of habituating them to manipulative disturbances, which included water deprivation, automotive transportation, confinement to individual irradiation chambers, and consumption of tap water under experimental conditions.

Approximately 24 hr. before neutron exposure, the animals were deprived of water to ensure consumption during the conditioning phase. On the following day they were transported to Berkeley (17 miles) for exposure to the cyclotron beam. Immediately prior to exposure a single plastic bottle was attached to each cage and the animal was allowed to drink during a 20-min. period. As specified by the experimental design summarized in Table 1, the bottles contained either tap water or a saccharin solution of 1 gm. per litre of water. Observations indicated that all the animals tasted the fluid at this time.

For exposure to fast neutrons, each animal was confined to a cardboard cylinder, 2½ in. in diameter by 7 in. in length. The 60-in. cyclotron supplied

Table 1. Experimental Treatment for Each Group with Respect to Radiation Dose and Type of Fluid consumed Prior to Exposure

Group	No. of animals	Neutron dose (rads)	Fluid consumed before exposure
I	14	0	tap water
II	16	0	saccharin solution
III	16	7·5	tap water
IV	15	7·5	saccharin solution
V	14	15	tap water
VI	15	15	saccharin solution

neutrons by bombarding a thick beryllium target with 12 MeV. protons. The mean neutron energy was approximately 2 MeV. The animals were placed at isodose distances, 30 in. from the target. Groups III and IV received 7·5 rads at 10 μamp. beam current while groups V and VI received 15 rads at 20 μamp. Both doses were delivered in 4 min. The animals were turned 180° at the mid-point of exposure to provide a more uniform distribution of dose. Dosimetry for field geometry and exposure dose was made by measuring the fast neutron flux with sulphur threshold detectors as described by Tochilin and Kohler[5]. Gamma background was approximately 12 per cent of the neutron dose under the conditions of exposure used. Groups I and II were sham-irradiated in a corridor outside the water tank shield which surrounds the cyclotron. The environment in the corridor was comparable to that of the radiation exposure room.

The post-irradiation test for conditioned behaviour began approximately 40 hr. after the neutron exposure. The water bottle on the home cage was replaced by two bottles; one containing tap water and the other containing saccharin solution. The amount of fluid consumed from each bottle for a 24-hr. period was measured and the saccharin preference score for each animal is expressed as the ratio:

$$\frac{\text{saccharin solution intake (gm.)}}{\text{total fluid intake (gm.)}} \times 100$$

The results of the saccharin preference test following the neutron exposure are summarized in Table 2. The animals which drank saccharin-flavoured water prior to fast neutron bombardment for 7·5 rads exhibited an 80 per cent reduction in preference for the fluid as compared to the control groups ($P < 0{\cdot}01$). The effect of the 15 rad dose was significantly greater than the 7·5 rad dose ($P < 0{\cdot}01$). Two-thirds of the saccharin group exposed to 15 rads drank less than 1 ml. from the saccharin bottle during the 24-hr. test, but drank normal amounts from their tap water bottle. The animals which consumed tap water before the exposure to fast neutrons drank approximately 90 per cent of their total fluid intake from the saccharin bottle; a preference which was comparable to that of the non-exposed controls.

Table 2. The Mean Saccharin Preference Score for Each Group during a 24-hr. Test commencing 40 hr. After a Single Exposure to Fast Neutrons

Neutron dose (rads)	Groups II, IV, VI (saccharin consumed before exposure)	Groups I, III, V (tap water consumed before exposure)
0	84·6 (4·4)*	90·6 (2·7)
7·5	15·4 (2·7)	89·9 (2·2)
15	4·7 (1·2)	87·3 (3·2)

* Standard error of the mean.

Neutron exposure is apparently an unpleasant experience which the rat associated with the taste of saccharin so that its post-irradiation preference for this substance is altered. The dose employed to establish this avoidance is relatively small; a single total-body dose of approximately 300 rads is required to produce deaths within 30 days post-exposure under the conditions employed in this study.

It may be concluded that fast neutrons as well as γ- or X-rays can be employed as an unconditioned stimulus to produce avoidance behaviour. Furthermore, in contrast to the previous studies which employed radiation exposures of several hours at low dose-rates, the neutron exposure lasted only a few minutes, indicating that the conditioning can be obtained under a wide variety of dose-rates and exposure conditions.

The opinions or assertations contained herein are those of the authors and are not to be construed as reflecting the official views of the Defense Department. We gratefully acknowledge the advice of E. Tochilin and B. W. Shumway concerning neutron dosimetry.

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Estimation of the Cellular Lethal Dose and the Critical Cell Number for the *C3H* Mouse Mammary Carcinoma from Radiosensitivity Studies *in vivo*

If it is assumed that irradiation of a tumour results in immediate or eventual sterilization of a given proportion of its constituent cells[1], that the magnitude of this effect is an exponential function of dosage[2,3], and that the ability of the residual viable cells to grow progressively depends upon whether their number exceeds that critical value below which their growth is effectively suppressed by host-resistance factors alone[4,5], then the radiosensitivity, or mean lethal dose, of the individual cells, and the critical cell number for the *C3H* adenocarcinoma growing under various conditions, can be estimated indirectly from observed data on the radiosensitivity of the tumour *in vivo*.

Taking the volume of a tumour cell to be of the order of 5×10^{-9} cm.^{3}, and allowing that the stroma and non-viable elements constitute roughly one-half of the tumour mass, we can safely assume each tumour cell to occupy a volume of 10^{-8} cm.^{3}, and accept this convenient unit as a standard 'cell' for the purpose of calculation. The validity of the following argument would not be affected if the actual mean tumour-cell volume differed somewhat from this assumed value.

If the rate of inactivation of tumour cells is exponential with dose, the residual cell population (A) after a given dose of radiation (D) is given by a function of the type:

$$A = N.e^{-kD} \qquad (1)$$

where N is the initial cell population, and k a cellular radiosensitivity constant characteristic of the tumour and equal to the reciprocal of the mean lethal dose (synonymous with the 37 per cent-survival dose or D_0).

Table 1 summarizes the results of a series of experiments on the radiosensitivity of the *C3H* mouse mammary carcinoma under various conditions, based upon a total of 799 individual treatments, and representing the most accurate estimates of the relevant parameters currently available[6,7]. The respective median lethal doses for the tumour treated *in situ* are 7,500 rads in immunologically incompetent hosts previously exposed to whole-body irradiation

Table 1. CONSOLIDATED DATA ON THE RADIOSENSITIVITY OF THE *C3H* MOUSE MAMMARY CARCINOMA IN VARIOUS SITUATIONS

Host–tumour relationship	Dose*	Proportion cured	Cure-rate (per cent)	Estimated *LD*50	Standard error	Estimated critical cell No.
Immunologically incompetent hosts	9,000	12/18	67	7,500	± 250	1
	7,500	25/50	50			
Standard isogenic situation	7,500	54/54	>98	5,660	± 40	25
	6,000	26/39	67			
	5,000	8/42	19			
	4,200	0/108	< 1			
Relatively 'immune' mice	5,000	12/26	54	4,800	± 100	200
	4,200	112/418	27			
	3,500	0/44	< 2			

* Rads : single exposure at 500 rads/min., 250 kVp. unfiltered, 2 cm. field at 25 cm. focus-skin distance, half-value 0·34 mm. copper.

or corticoid hormones[7], 5,660 rads in the standard isogenic host–tumour relationship[6], and 4,800 rads in mice rendered relatively resistant to the tumour by various 'immunizing' procedures[6,8]. In the case of tumour fragments irradiated prior to implantation (revised data[4] not included in Table 1), the dose yielding 50 per cent 'takes' was found to be 3,250 (± 110) rads.

From the data given it appears that the dose required to cure 37 per cent of tumours under conditions of minimal host resistance is about 7,000 rads, at which dose at least 1 viable cell must have persisted, on the average, for each tumour treated, that is, $A \ll 1$. Since these tumours were irradiated when they had reached a diameter of 1 cm. (volume 0·5 cm.^{3}), the initial cell population $N = 0{\cdot}5 \times 10^8$. Substituting in equation (1), it follows that k cannot be greater than 0·00254 rad^{-1}, and that D_0, the cellular mean lethal dose, must be at least 394 rads.

An alternative estimate of this parameter can be obtained from the relative radiosensitivities of tumours (500 mm.^{3}) treated *in situ* in unmodified hosts ($LD50 = 5{,}660 \pm 40$ rads), compared with tumour fragments (1 mm.^{3}) irradiated before implantation ($LD50 = 3{,}250 \pm 110$ rads). If the dosage difference, $\Delta D = 2{,}400$ (± 120) rads, is entirely attributable to the 500-fold difference in cell-population, then $e^{-\Delta D/D_0} = 1/500$, and $D_0 = 385$ (± 20) rads. Since the two estimates are in close agreement, one could take the mean lethal dose for *C3H* carcinoma cells to be of the order of 390 rads.

From the foregoing data, the critical cell number, just permitting continued tumour growth in the mouse, can be computed. The median tumour lethal dose of 5,660 rads for 500 mm.^{3} growths treated *in situ* corresponds to a residual viable cell population of $0{\cdot}5 \times 10^8/e^{(5{,}660 \pm 40)/390} = 25$ (± 3) 'cells'. Similarly, for the 1 mm.^{3} fragments irradiated prior to implantation, the dose of 3,250 rads corresponds to a viable cell population of $10^5/e^{(3{,}250 \pm 110)/390} = 25$ (± 7) 'cells'. A reasonable estimate, therefore, of the critical cell number, permitting 50 per cent growths in the normal *C3H* mouse bearing the standard isogenic mammary tumour, would be of the order of 20–30 cells.

In the group of relatively 'immune' mice, the estimated $LD50$ of 4,800 rads corresponds to a surviving population of more than 200 'cells'.

The cell-population concept, however, cannot in itself account fully for the variation in cure-rate with dosage. Given the average cell population for tumours treated at a particular dosage, factor A in equation (1), the proportion of cured tumours (P) presumed to contain less than Z cells (the critical number), is given by a Poisson exponential summation:

$$P = e^{-A} \sum_{x=0}^{z-1} \left(\frac{A^x}{x\,!} \right) \qquad (2)$$

Comparison of this function with the slope of the empirical 'probit' regression for the data shows the latter to be significantly greater. This difference reflects the variance of the observations, attributable in the main to the inherent dosimetric error, accounting for a 10 per cent coefficient of variation, and in part to minor genetic variation among individual mice.

In summary, the minimum number of cells capable of progressive growth in the three types of host–tumour relationship studied, that is, in the immunologically incompetent, the normal, and the 'immune' states, are of the order of 1, 25 and 200 tumour cells respectively. Assuming a constant radiosensitivity of the cells *per se*, the dose of radiation which is just sufficient to deplete the tumour cell population to the critical level will vary with different degrees of host resistance. Changes in radiosensitivity associated with various host–tumour combinations can, therefore, be accounted for in terms of differences in the corresponding critical cell assemblies.

The possibility that a low oxygen tension had raised the apparent lethal dose in our experiments cannot be excluded, although there is evidence that anoxia was not complete and that a similar degree of oxygenation existed both in the tumour treated *in situ* and in the fragments irradiated before implantation (unpublished data). Since complete anoxia may increase the inactivation dose by a factor of about 3[1], it seems unlikely that our estimate of D_0 would need to be reduced by much more than a factor of 2. On the other hand, consideration of the relative biological efficiency of the relatively soft radiation used in our experiments (half-value layer, 0·34 mm. copper) might necessitate an upward revision of the estimated D_0. Thus the mean lethal dose for the *C3H* carcinoma appears to be substantially greater than that reported for certain other tumours by more direct methods[2,3].

The foregoing reasoning is naturally based upon a quantal response to irradiation, namely, the lethal effect on tumour cells. It fails to account for the 'immunizing' effects induced by radiation-attenuated isogenic inocula, or for the graded reactions and interactions appearing in many tissues, organs and animals, which have been collectively described in terms of 'antigenic equivocation'[9]. It seems probable that the 'equivocation' and 'critical assembly' hypotheses are complementary. A detailed report on these experiments will be published elsewhere.

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An Autoradiographic Study of the Distribution in Mice of Œstrogens labelled with Carbon-14 and Tritium

We have examined the distribution in mice of the metabolic products of two œstrogens by an autoradiographic method which allows histological localization. It is of interest to use a 'physiological' dose, which Dodds[1] estimates to be close to 1 μgm. hexœstrol in the rat. Glascock and Hoekstra[2], using tritium-labelled hexœstrol in the goat and the sheep, found that the maximum quantity of the injected product which could be recovered did not at any time exceed 0·2 per cent in the uterus or 0·05 per cent in the vagina.

In preliminary experiments, we used œstrone-16-^{14}C of specific activity 2·7 μc./mgm., and in a second series œstradiol-17β-6,7-^{3}H (from C. E. Frost Co., Montreal, and the New England Nuclear Corp., Boston, respectively, and supported by a grant from the Fondation Fontaine), of specific activity 3·85 mc./mgm. The doses were respectively 1 mgm. œstrone-16-^{14}C (2·7 μc.) dissolved in poppy-seed oil and 50 μgm. œstradiol-17β-6,7-^{3}H (200 μc.) dissolved in olive oil, per animal. Female mice, weighing 25–30 gm., were injected at arbitrary stages in the œstrous cycle. The animals were killed after ½, 1½, 4 and 20 hr. in the first series, and after 3, 4, 6 and 12 hr. in the second. Fragments of the uterine tubes, the kidneys and the liver, and the whole of the ovaries were taken out. After fixation for 4 hr. in 10 per cent formaldehyde, two series of biopsies were prepared: the first was washed in water for 1 hr., the other for 12 hr. Because paraffin embedding was unsuitable, owing to the solubility of œstrone and œstradiol in alcohol, sections (20μ) were cut from material frozen with carbon dioxide. The sections were spread on gelatin-covered slides and treated for track autoradiography with nuclear emulsion Ilford *G* 5[3]. They were exposed for 6–10 days, developed and then stained with 'Unna'. Uninjected mice and mice injected with the solvent oils were treated similarly.

The results of the first series were entirely negative (œstrone-16-^{14}C). We observed no localization of β-tracks which could be attributed to the injected material. This failure is not surprising considering the small amount of radioactive material injected (2·7 μc.) and the fact that œstrogens are rapidly metabolized and excreted by the kidneys and the bile ducts[4].

In the second series (œstradiol-17β-6,7-^{3}H) when the animal had been killed after a short time (3–4 hr.) a localization of grains was observed in the kidneys and in the uterine tubes. These grains have the characteristic appearance of those produced by tritium β-rays. In the uterus, these grains are found in the cells of the endometrium and in the lumen of the glandular tubes, in contact with the apical pole of cells (Fig. 1), from which they are not removed by prolonged washing. However, Rengler[5] reports that, in rats injected with œstrone-16-^{14}C, the major portion of the radioactivity of the fluid from washings of the uterine lumen is present as a water-soluble component and that no radioactive steroids can be recovered from this fluid. Such a localization of grains was not observed after 6–12 hr.

In the kidneys, the grains are found in the periglomerular space and in the first part of the convoluted tubule, after 3–4 hr. (Fig. 2); but they are eliminated by prolonged washing. Similarly, after 6–12 hr., they are scarcely observable. This radioactive material, localized in the first part of the nephron, is therefore probably soluble, and seems to be filtered through the glomeruli. Budy[4] reports that the concentration of radioactive product recovered from the kidney is higher than one would expect, judging from the radioactivity of the urine; she attributes this result either to an effect of the blood, or to a re-absorption by the convoluted tubule. Our results favour the latter view.

Fig. 1. Grains in the endometrial cells and in the lumen of the glandular tubes

In the liver and ovaries we have not observed any distribution of radioactivity which could be correlated with the structure of the tissues. It has been known for a long time that the liver plays an important part in the metabolism and the excretion of the œstrogens, and the absence of systematic localization might be related to the special histological structure of this organ, or to the moment when the animal was killed.

In the ovary, the lack of radioactivity in the present experiments is more difficult to understand, since Glascock[2] has found in this organ, for the same weight, half the radioactivity observed in the uterus.

The control sections were negative. One must, however, point out the presence of artefacts—fine grains and lines where the sections are disrupted.

In conclusion, the histological localization of the metabolic products of tritium-labelled œstrogens seems to be possible. Investigations with a more 'physiological' dose and examination within 2 hr. of injection should not be without interest.

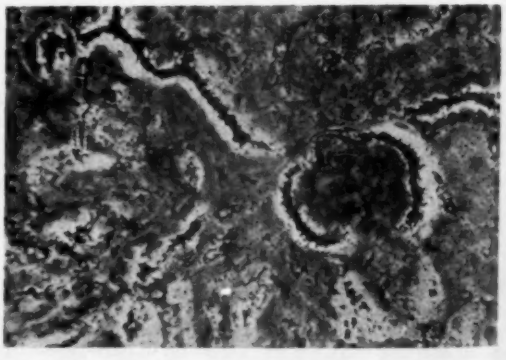

Fig. 2. Numerous grains in the periglomerular space and in the first part of the convoluted tubule

I wish to thank Prof. J. Brachet, who allowed me to study this question in his laboratory, and I am particularly indebted to Dr. A. Ficq for her advice and kind technical co-operation.

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BACTERIOLOGY

Interspecific Hybridization among *Mycobacteria*

Experiments conducted in the first part of this work showed that a high degree of streptomycin resistance can be transmitted to *M. phlei* when a streptomycin-sensitive strain of this micro-organism and a streptomycin-resistant *BCG* strain of *M. tuberculosis* are cultured together in 'Tween' liquid medium.

Resistance to 50 μgm. streptomycin per ml. (S^r) was selected as marker in the *BCG* strain; the markers selected for *M. phlei* were pigment formation (Pig^+) and the capacity for growth on plain agar medium (Ag^+). The *BCG* strain may thus be described as S^r Pig^- Ag^-, while *M. phlei* is S^s Pig^+ Ag^+. Colonies of the new organism were picked from a selective medium: tryptose agar containing 50–1,000 μgm. streptomycin per ml. on which neither parent organism could grow when planted alone. Their inability to grow on this medium in 30 or more days served as control. The frequency of resulting streptomycin-resistant organisms was estimated to be 1–10 per 10^8 *M. phlei* units. Similar results were obtained in four experiments.

One of the strains obtained in the course of these experiments deserved special attention as it resembled the so-called 'atypical' acid-fast chromogenic *Mycobacteria*. This hybrid, in addition to high streptomycin resistance, showed yellow pigmentation (both in the dark and in the light) which, in contrast to that of the parental *M. phlei*, turned orange on prolonged cultivation. At 37° C. the strain grew on tryptose agar, as well as on 'Tween' agar or Loewenstein's medium within 5–12 days; at room temperature growth appeared slowly, in 12–20 days. No cord formation was seen. A high degree of pleomorphism and instability of traits other than the selected markers were found to be characteristic.

Since streptomycin was the only selected marker of the *BCG* strain used in these experiments, it was not possible to make any recombination analysis, or to assess the importance of a cell-to-cell contact (conjugation) in the transfer of streptomycin resistance.

Experiments were, therefore, carried out in order to shed more light on the mechanism of transfer: *M. phlei* was grown in the culture filtrate of the *BCG* strain; and *BCG* in the culture filtrate of *M. phlei*. Cell-free filtrates of *M. phlei* were never observed in these experiments to bring about changes in the *BCG* strain. Sterile culture filtrates of the *BCG* strain, on the other hand, conferred the streptomycin resistance to *M. phlei* cells. This could be repeated in three experimental series. The frequency, however, was 10^2 lower, that is, 1–10 per 10^6 *M. phlei* units. These findings indicate that the transfer of streptomycin resistance does not necessarily require cells as donors, and thus transduction may be the actual mechanism of transfer. (In the trials already carried out deoxyribonuclease treatment of the filtrate has not affected significantly its activity.)

These experiments, both those in which a *BCG* culture and those in which cell-free filtrates of a *BCG* culture were used as donors, disclosed also that: (1) The streptomycin resistance of descendant organisms increased to a level of more than 1,000 μgm. per ml. as against a level of 50 μgm. per ml. of the parental *BCG* strain of *M. tuberculosis*. (2) This high level of streptomycin resistance was attained in a single step, while the parent strain of *BCG* acquired the much lower resistance in four or five distinct steps. (3) In addition to this, a considerable portion of the resulting variants showed on isolation dependence on streptomycin. (4) Evidence was also obtained supporting the assumption that the transfer of hereditary traits is uni-directional, that is, from *BCG* strain of *M. tuberculosis* to *M. phlei*.

The results of the work described here appear to bear a resemblance to the findings made in the course of investigations of genetic transfer in various species of bacteria[1–5]. Studies on genetic transfer together with certain recently reported observations with respect to mutation[6–9] may, it is hoped, some day give an answer to the origin of atypical mycobacteria.

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Role of *Hfr* Mutants in *F+* × *F−* Crosses in *Escherichia coli* K12

Genetic recombination between mutant strains of *Escherichia coli* strain *K12* is governed by a system of mating types[1,2]. The first two mating types to be discovered were called *F+* and *F−*. In crosses the *F+* parent donates genetic material to the *F−* parent. The progeny are predominantly *F+*. At the same time it was discovered that the *F* factor was infectious, that is, if *F+* and *F−* strains were mixed and the organisms of the *F−* strain re-isolated, a proportion had become *F+*. It was therefore possible that the *F+* state of the progeny was secondary and that any *F*-zygotes became infected by other *F+* cells.

Then came the discovery of mutant strains of a new mating type known as high-frequency recombining (*Hfr*) strains[1,2]. Whereas *F+* strains crossed with *F−* strains gave only one recombinant per

Table 1. Details of Four Experiments on Secondary Infection by *F* Factor after Recombination

Experiment	*F*− cells per plate	*F*+ cells per plate	Recombinant colonies per plate in first half	Percentage of colonies in first half *F*+	*Hfr* cells added per plate for second half	Recombinant colonies per plate in second half	Percentage of colonies in second half *F*+	Calculated percentage of zygotes in first half originally *F*+
1	$2{\cdot}5 \times 10^8$	$2{\cdot}5 \times 10^7$	n.r.	95	$2{\cdot}5 \times 10^6$	n.r.	36	92
2	$2{\cdot}5 \times 10^8$	$2{\cdot}5 \times 10^6$	25	80	10^6	600	14	77
3	$2{\cdot}5 \times 10^8$	$2{\cdot}5 \times 10^7$	150	77	10^5	2,000	44	60
4	$2{\cdot}5 \times 10^8$	$2{\cdot}5 \times 10^6$	4	96	10^4	250	21	95

n.r., not recorded

10^3 parents, these *Hfr* strains when crossed with *F*− strains gave about 1,000 times this number of recombinants. The progeny of such crosses were *F*−. *F*+ organisms apparently mutate to the *Hfr* state and *Hfr* clones can be isolated from *F*+ cultures[4].

Jacob and Wollman suggested that these mutant forms were the agents in recombination in *F*+ × *F*− crosses, and that the *F*− progeny so formed were then secondarily infected by the *F*+ cells present. In support of their theory they measured the fluctuation in the number of recombinants obtained from several small *F*+ cultures. This suggested that the recombination was at least in part due to clones of *Hfr* cells. However, this type of evidence does not prove conclusively that cells of such clones are the only donors of genetic material in an *F*+ culture, and Lederberg has suggested that they are not[5]. Some experiments were designed to show whether secondary infection can, in fact, account for the high proportion of *F*+ progeny found in an *F*+ × *F*− cross.

In these experiments we have used *K*12 mutants 58–161/*F*+ (requiring methionine), *W*677/*F*− (requiring threonine, leucine and aneurin) and *Hfr* strains isolated from 58–161/*F*+. Washed log-phase broth cultures of these strains were suspended at approximately $2 - 5 \times 10^8$ viable organisms/ml. Each experiment consisted of two halves. In the first half the 58–161/*F*+ suspension was mixed with the *W*677/*F*− suspension in a proportion varying from 1 : 10 to 1 : 100. Aliquots of 0·5 ml. of this mixed suspension were immediately spread over each of several plates of minimal agar plus aneurin. The liquid was very quickly soaked up. For the second half of the experiment small numbers of *Hfr* cells were added to the mixed suspension before dispensing on to the plates. The actual numbers are given in Table 1. Both series of plates were incubated 36 hr. and the number of colonies counted. Neither parent could grow on this medium and the colony counts gave an indication of the recombination-rate.

Single colonies from these plates were streaked out on the same medium to obtain colonies free of parental cells and single colonies from these streaks tested for their mating type by crosses with *W*677/*F*− (streptomycin resistant). Those which would cross were recorded as *F*+ and those which did not as *F*−. In experiments 3 and 4 the colonies scored as *F*− were shown not to be chance contaminants by their ability to cross with 58.161/*F*+ (streptomycin resistant) but not with *W*677/*F*− (streptomycin resistant).

In each experiment the addition of the *Hfr* suspension increased the recombination-rate several-fold and therefore most of the progeny in the second half, having come from *Hfr* parents, must have been originally *F*−. The proportion of *F*+ progeny in the second half gives us, then, the rate of secondary infection under these experimental conditions. If it is assumed that the probability of secondary infection is the same in both halves of the experiment we can calculate the proportions of the zygotes in the first half which were originally *F*− and *F*+. This is given in the last column.

While prior mutation to *Hfr* undoubtedly accounts for some of the recombinants in an *F*+ cross, the proportion which are formed via *Hfr* mutation is not certain.

The existence of a significant proportion of *F*+ zygotes in the first half of the above experiment makes it evident that the genetic recombination in an *F*+ × *F*− cross is not mediated entirely by stable *Hfr* mutants of the type so far isolated, but that some *F*+ cells cross directly with *F*− cells.

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Synthetic Polypeptide Derivatives with Anti-Bacterial Activities

A number of polypeptides composed of various basic amino-acids have been prepared by Katchalski *et al.*, and it had also been stated that agglutination of Gram-positive and Gram-negative bacteria was caused by these polypeptides, and bacteriostatic or bactericidal activity was observed depending on their concentration[1]. Stahmann and his co-workers[2] have reported that the multiplication of some viruses was restrained by poly-L-lysine.

From these observations and the fact that a number of naturally occurring antibiotics (for example, tyrocidine and polymyxins) have peptide structures and basic characters, it may be assumed that the microbiological activity of these compounds depends fundamentally on these structures and characters.

The synthetic polypeptides composed of basic amino-acids, however, cannot be employed for pharmacological use for they are soon disintegrated by the proteolytic enzymes of the higher organisms.

As previously stated[3], polymethyl-α-poly-L-glutamate can be converted with ethylenediamine into a water-soluble peptide derivative ('Polyanthin') which is resistant to proteolytic enzymes of allantoic liquid and at the same time efficiently hinders the development of bacteriophages T_3, T_4 and T_7. Its sub-

cutaneous toxicity is about $LD50 = 850$ mgm./kgm. in mice.

The possible anti-tuberculous activity of this product has been investigated as follows (the 'Polyanthin' used in the experiments was kindly supplied by the Chinoin Chemical and Pharmaceutical Works Co., Budapest):

(1) To 1 ml. of a solution of 3,000γ 'Polyanthin' 0·01 mgm. (wet weight) of a three-weeks-old *H37Rv* culture was added. One part of the suspension was stored at room temperature, the other part at 37° C. Samples of 0·1 ml. were taken at different intervals and inoculated on Löewenstein–Jensen and Sula media. Other samples of 0·1 ml. were injected into pairs of guinea pigs. The culture media were examined after six weeks incubation at 37° C., while the animals were submitted to section and examined after eight weeks. Both results are summarized in Table 1.

Table 1. Relation of 'Polyanthin' Activity to Time and Temperature of Application against *Mycobacterium tuberculosis*

No. of experiment	Time (hr.)	Temperature (° C.)	No. of colonies	Degree of tuberculosis at autopsy
I*a*	16	37	many	generalized
I*b*	16	room	many	generalized
II*a*	24	37	many	generalized
II*b*	24	room	many	generalized
III*a*	72	37	10	moderately generalized
III*b*	72	room	many	moderately generalized
IV*a*	120	37	5	Only changes in regular lymphatic glands, and a few tubercules in spleen
IV*b*	120	room	4	
Control*	120	37	many	generalized
Control	120	room	many	generalized

* The controls were suspensions of tubercle bacilli in physiological saline solution.

The results show that 'Polyanthin' exhibits a bacteriostatic activity, increasing with the time of application.

(2) Thirty guinea pigs (about 350 gm.) were infected subcutaneously with 0·001 mgm. (wet weight) suspensions of *H37Rv*-Sula culture. (The suspension was prepared with rust-proof steel balls.) To each of 20 guinea pigs, once, in a single dose, a neutral solution of 60 mgm. 'Polyanthin' was given subcutaneously on the day following the infection. Ten guinea pigs were left for control. After 93 days the animals were submitted to autopsy. The degree of tuberculous change found in the different organs was estimated in whole numbers in the range of 0–4, and mean values were calculated. The following organs were examined: regulare lymphatic gland, retroperitoneal lymphatic gland, spleen, liver and lungs. The index for the control group was 3·4 and for the other group that got the 'Polyanthin' injection 1·4.

These experiments seem to show that 'Polyanthin' exhibits considerable anti-tuberculous activity. Further experiments are in progress.

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Cystine as an Essential Amino-Acid for the Growth of a Strain of *Shigella dysenteriae* I (*Sh. shiga*)

In the studies on the nitrogen requirements for the growth of members of the genus *Shigella* in a chemically defined medium with various nitrogen sources added singly[1] a strain of *Shigella dysenteriae* 1 was found to grow only when cystine was present.

The basal medium consisted of 5 ml. each of salt solutions *A* and *B*[2], glucose 0·1 *M* and nicotinamide 10^{-5} *M*. To this was added, singly, the following: ammonium chloride (1 per cent), DL-glutamic acid, DL-phenyl alanine, DL-histidine, DL-lysine, DL-threonine, glycine, l-cystine, l-cysteine hydrochloride, DL-leucine, l-arginine, DL-aspartic acid, DL-alanine, DL-tryptophan, DL-valine, DL-*iso*leucine, DL-methionine, DL-tyrosine, DL-proline and DL-serine. The various nitrogen sources were dissolved in the basal medium with gentle heating. Cystine required the aid of hydrochloric acid and for tyrosine *N* sodium hydroxide was needed[3]. The amino-acids were present at 0·01 *M* concentration, except cystine and tyrosine, which were used at 0·0005 *M* and 0·002 *M* concentrations, respectively. The final *p*H of the medium was adjusted to 7·2–7·4; the medium was sterilized by autoclaving at 115° C. for 15 min. The inoculum of the organism was 0·1 ml. of an overnight peptone water growth diluted one in a hundred in sterile double-distilled water. Incubation was carried out aerobically at 37° C. and continued for seven days. If growth occurred during this period, one loopful of the growth was transferred to a second tube of the same medium. The nitrogen source was recorded as giving positive growth if three serial sub-cultures showed growth of the organism.

Cystine was the only amino-acid, among the nitrogen sources tested, in which growth of the strain could be obtained. As cysteine did not produce growth, further experiments were conducted and the results are shown in Table 1. The possibility of impurities in the cystine originally used was explored by using a preparation from another source of supply. The result was as before. The possibility that autoclaved cysteine, by producing colloidal sulphur and colloidal copper sulphide[4], may have caused inhibition of growth was eliminated by using filtered cysteine[5]. An attempt to grow the organism in cysteine containing medium which had been shaken aerobically at 37° C. for 6 hr., in order to abolish inhibition of growth by exogenous cysteine[6], was unsuccessful. There is a possibility that insufficient amounts of cystine were formed from oxidation of cysteine, for in a medium containing 0·02 mgm. of cystine per ml., as used by Proom in the study of the nutrition of Providence strains[7], the strain of *Sh. dysenteriae* 1 did not show any growth.

Johnson[8] suggested that cystine had a nutritional role for a strain of *Sh. dysenteriae* 1 investigated by him. The results of the present investigation in

Table 1. Growth of *Sh. dysenteriae* 1 in Medium containing Cystine and Cysteine

Medium containing inorganic salts, glucose, nicotinamide and the following	Growth
1 Cystine, 0·0005 *M*	+
2 Cysteine, 0·01 *M*	−
3 Cysteine, 0·0005 *M*	−
4 Cysteine, 0·01 *M* — Medium Seitz filtered	−
5 Cysteine, 0·0005 *M* — Medium Seitz filtered	−
6 Glutamic acid, 0·01 *M* + cystine, 0·0005 *M*	+
7 Glutamic acid, 0·01 *M* + cystine, 0·02 mgm. per ml.	−
8 Glutamic acid, 0·01 *M* + cysteine, 0·01 *M*	−
9 Glutamic acid, 0·01 *M* + cysteine, 0·0005 *M*	−

Table 2. GROWTH OF *Sh. dysenteriae* 1 IN MIXTURES OF AMINO-ACIDS WITH AND WITHOUT CYSTINE. CYSTINE, WHEN PRESENT, WAS AT 0·0005 *M*; ALL OTHER AMINO-ACID CONCENTRATIONS WERE 0·01 *M*

	Medium consisted of basal medium and the following amino-acid mixtures:	Growth
1	Glut. + meth. + tryp. + asp. + cys.	+
2	Glut. + meth + tryp. + asp.	−
3	Glut. + meth. + tryp. + cys.	+
4	Glut. + meth. + tryp.	−
5	Glut. + asp. + tryp. + cys.	+
6	Glut. + asp. + tryp.	−
7	Glut. + asp. + meth. + cys.	+
8	Glut. + asp. + meth.	−
9	Asp. + meth. + tryp. + cys.	+
10	Asp. + meth. + tryp.	−
11	Glut. + tryp. + cys.	+
12	Glut. + meth. + cys.	+
13	Glut. + asp. + cys.	+
14	Meth. + tryp. + cys.	+
15	Glut. + tryp.	−
16	Glut. + meth.	−
17	Glut. + asp.	−
18	Glut. + cys.	+
19	Meth. + tryp.	−
20	Tryp. + asp.	−
21	Tryp. + cys.	+

Glut., glutamic acid; meth., methionine; tryp., tryptophan; asp., aspartic acid; cys., cystine.

showing that growth of the strain occurred only when cystine was present in mixtures of amino-acids, as tabulated in Table 2, would also favour an essential nutritional role for the amino-acid for this particular strain of *Sh. dysenteriae* 1.

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VIROLOGY

Persistence of Herpes Simplex Virus in HeLa Cells

PERSISTENCE of herpes virus in cells cultured *in vitro* might provide a suitable model for investigating the phenomenon of latent herpes infection in humans. During a study on the interaction between herpes virus and HeLa cells, it was observed that an extensive cytopathic effect eventually resulted from inoculation of a monolayer culture with the *HFEM* strain of herpes virus[1,2]. A few isolated cells sometimes survived, however, and if growth medium containing antiviral serum was added, colonies grew from these surviving cells, leading eventually to a large population. These recovered cells were apparently healthy and grew as well as the original HeLa cells: there were no gross morphological differences such as those described by Vogt and Dulbecco[3], who studied cells recovered after poliomyelitis infection.

Despite continued growth in the presence of high-titre antiserum, however, the recovered cells were found to carry herpes virus for at least nine months, involving 29 subcultures. The presence of the virus was indicated by the appearance of typical microscopic foci of infected cells or plaques, from which virus could be isolated. These plaques appeared whether or not there was antiserum in the medium. When antiserum was present, however, the plaques remained localized and did not increase in number. It was therefore possible to deduce that there was one plaque-forming unit (presumably an infected cell) in approximately 10^5 cells. If antiserum was removed from the medium, the foci became larger and eventually confluent, with release of infective virus into the medium. No infective virus has been isolated from medium or cells at times when no plaques were visible.

By taking a small population of recovered cells it was possible to grow a sub-line which did not give rise to plaques spontaneously, even in medium free from antiserum. This sub-line was compared with the original HeLa cells for susceptibility to virus recovered from a single plaque in the recovered cells and then grown in chick cells to form a virus stock. With a large dose of virus, the virus-free recovered cells underwent a confluent cytopathic effect with release of virus. Comparative infectivity titrations in the recovered cells and the HeLa cell stock indicated, however, that 10–100 times as much virus had to be present to infect a recovered cell as that required to infect a stock HeLa cell.

The mechanism of persistence of herpes virus in recovered cells has yet to be elucidated; but the susceptibility of these cells to virus, although reduced, does not suggest a situation resembling temperature bacteriophage, and it is also unlikely that auto-interference of the type studied by Henle *et al.*[4] could be the explanation. It is possible that persistence is due simply to the very slow spread of virus in the selected population of less-susceptible cells in the presence of inhibitory medium. This would resemble persistence of poliomyelitis virus in partially resistant HeLa cells, studied by Ackermann and Kurtz[5] and Vogt and Dulbecco[3]. Unlike the poliomyelitis-infected cultures, however, the recovered cells continued to carry herpes virus in the presence of strong antiviral serum. This is in keeping with the known ability of this virus and the related *B* virus of monkeys to spread between contiguous cells in antiserum[6,7].

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, January 25

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. M. Paskins: "Transistors in Switching Circuits".

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Mr. J. C. G. Gilbert and Mr. R. S. Roberts: "The Problems of Technical Reviewing".*

Tuesday, January 26

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. R. W. R. Rutland: "Radioactivity in the Earth's Crust".*

UNIVERSITY OF LONDON (at Queen Mary College, Mile End Road, London, E.1), at 1.30 p.m.—Prof. J. D. Bernal, F.R.S.: "The Liquid State".*

UNIVERSITY OF LONDON (at the Imperial College of Science and Technology, South Kensington, London, S.W.7), at 1.30 p.m.—Mr. Ernest Gellner: "The Barbarian Scientist ?"* (Further lectures on February 2, 9, 16 and 23.)

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Dr. R. Maclagan Gorrie: "Irrigation and Population in Pakistan, India and Ceylon".

INSTITUTE OF MARINE ENGINEERS (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. R. Anscomb and Mr. F. Hutber: "Control and Instrumentation of Marine Reactors".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. J. Brachet (Université Libre de Bruxelles): "The Rôle of Ribonucleic Acid in Protein Synthesis".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. J. V. Pulvertaft: "Auto-Immune Reaction".* (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation. Further lectures on January 28, February 2, 4, 9, 11, 16, 18, 23, 25 and March 1.)

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. F. Buckley: "Polymerization and Copolymerization of Itaconic Acid and its Derivatives".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Symposium on "Flame Failure Detection". Mr. D. H. Surgeoner: "A Review of Current Flame Failure Devices"; Mr. D. Richards: "Applications of Flame Failure Equipment".

Wednesday, January 27

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 2 p.m. and 5 p.m.—Symposium on "Geological Implications of Recent Geophysical Work in the British Isles".

BRITISH COMPUTER SOCIETY, LONDON BRANCH (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 2.30 p.m.—Dr. G. G. Macfarlane: "Storage Elements for Very High Speed Computers".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. A. Clow: "The Oral Presentation of Scientific Material".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. J. Brachet (Université Libre de Bruxelles): "The Rôle of the Cell Nucleus in Ribonucleic Acid and Protein Synthesis".*

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—205th Scientific Meeting. Mr. W. H. Dawbin: "Whales and Whaling in the South Pacific Ocean".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. J. J. McMullen: "Technical and Economic Aspects Covering the Ocean Transportation of Liquid Methane" (Thomas Lowe Gray Lecture).

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Dr. K. R. Sturley and Mr. A. E. Robertson: "Training for Operating and Maintaining Television Broadcasting Equipment".

Thursday, January 28

CHALLENGER SOCIETY (joint meeting with REPRESENTATIVES FROM THE MARINE LABORATORIES (Development Commissioners' Scheme), in the Lecture Theatre, British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.30 a.m. and 2.30 p.m.—Scientific Papers.

INSTITUTION OF MINING ENGINEERS (at the Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 11.15 a.m.—66th Annual General Meeting.

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. Wm. T. Stearn: "How Many Species of Flowering Plants have Taxonomists Yet to Describe ?"; Dr. W. S. Lacey: "A Botanical Visit to Central and South Africa".

MINERALOGICAL SOCIETY (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. A. W. Lines: "Radio Communications by Means of Satellites".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. G. White: "Immune Cellular Reactions".* (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation. Further lectures on February 2, 4, 9, 11, 16, 18, 23, 25 and March 1.)

UNIVERSITY OF LONDON (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. P. Blanquet (University of Bordeaux): "Ion Exchange Resins and Thyroid Metabolism". (Further lecture on January 29.)

Friday, January 29

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. B. Dyott and Mr. C. R. Russell: "Beam-Type Parametric Amplifiers—Some Aspects of Design and Use".

UNIVERSITY COLLEGE (in the Eugenics Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. L. Lehmann (University of California): "The Efficiency of Non-Parametric Tests". (First of two Special University Lectures on Statistics. Further lecture on February 1.)*

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. B. S. Shenstone: "Engineering Aspects of Man-Powered Flight".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. Edgar Wind: "The Academy of Leonardo Da Vinci".

Saturday, January 30

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. G. H. Francis: "Exploration in the Southern Andes of Peru".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH OFFICER or RESEARCH CADET (with an honours degree in agricultural science, and preferably special training or experience in soils research) IN THE SOILS DIVISION (Soil Physics Department)—The Director, An Foras Talúntais, 33 Merrion Road, Dublin (January 29).

LECTURER and an ASSISTANT LECTURER (honours graduate in physics or chemistry) IN TEXTILE CHEMISTRY—The Registrar, The Manchester College of Science and Technology, Manchester 1 (January 30).

SENIOR TECHNICIAN IN THE NEW DEPARTMENT OF MEDICINE, to take technical charge and assist with biochemical research—The Secretary, Royal Free Hospital, Gray's Inn Road, London, W.C.1 (February 1).

RESEARCH FELLOW IN THE DEPARTMENT OF PHYSICS, for theoretical work in connexion with investigations now proceeding in the Department into the properties of solids at extreme infra-red and submillimetre wave-lengths—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (February 6).

UNIVERSITY DEMONSTRATOR (with an appropriate honours degree and preferably postgraduate research or teaching experience) IN ANIMAL PHYSIOLOGY—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (February 6).

JUNIOR LECTURER IN PHYSIOLOGY—The Registrar, Trinity College, Dublin (February 8).

LECTURER or ASSISTANT LECTURER IN STATISTICS—The Registrar, The University, Leeds 2 (February 8).

LECTURER (preferably with a veterinary qualification) IN PHARMACOLOGY in the School of Veterinary Medicine—The Registrar, Trinity College, Dublin (February 8).

LECTURER of ASSISTANT LECTURER IN THE DEPARTMENT OF GENETICS, to assist chiefly with biometrical and population genetics—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (February 10).

ASSISTANT LECTURER or LECTURER (with special interests in inorganic chemistry) IN CHEMISTRY—The Registrar, The University, Sheffield (February 13).

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF ELECTRICAL ENGINEERING (ELECTRONICS)—The Registrar, The University, Liverpool, quoting Ref. CV/T (February 13).

CHAIR OF PHYSICS—The Registrar, The University, Southampton (February 15).

LECTURER or ASSISTANT LECTURER IN PURE MATHEMATICS—The Secretary and Registrar, The University, Southampton (February 15).

LECTURER or ASSISTANT LECTURER (with a first-class degree in mathematics and a strong interest in research in some field of applied mathematics) IN APPLIED MATHEMATICS—The Secretary and Registrar, The University, Southampton (February 15).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (PHYSICAL CHEMIST) (with a first- or second-class honours degree in physical chemistry or equivalent), at Warren Spring Laboratory, D.S.I.R., Stevenage, Herts, to work on application of radio isotopes in research problems being pursued in the laboratory—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting reference F.872/9A (February 15).

CHAIR OF BOTANY at Westfield College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 19).

CHAIR OF CHEMISTRY at Westfield College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 22).

Chair of Mathematics at Westfield College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 23).

Scientific Officer (with a first- or second-class honours degree in chemistry) in the Department of Pedology, Forest Soils Section, for duties mainly concerned with studies on nitrogen in peat in relation to afforestation, as well as the development and application of methods for foliage analysis—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (February 27).

Assistant Lecturer or Lecturer in Organic Chemistry—The Secretary and Registrar, The University, Southampton (February 29).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry, Engineering, Pharmacology or Physics—The Secretary of University Court, The University, Glasgow (February 29).

Lecturer or Assistant Lecturer in Organic Chemistry—The Secretary and Registrar, The University, Southampton (February 29).

Assistant Lecturer in Chemistry—The Secretary, The University, Exeter (March 1).

Assistant Lecturer (preferably with an interest in theoretical plasma physics) in Physics—The Secretary, The Queen's University, Belfast (March 1).

Lecturer (with experience in one of the following fields: general physical metallurgy, metallurgy of surfaces, high temperature materials and ceramics) in the Department of Physical Metallurgy—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (March 1).

Imperial Chemical Industries Research Fellows in Chemistry, Civil Engineering or Physics—The Registrar, Trinity College, Dublin (March 7).

Imperial Chemical Industries Fellows for Research in Physics, Chemistry, Colloid Science, Biochemistry, Engineering, Metallurgy, Pharmacology, Chemotherapy, or related subjects—The Secretary-General of the Faculties, The University, The Old Schools, Cambridge (April 30).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry, Chemotherapy, Engineering, Metallurgy, Pharmacology or Physics—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 30).

Turner and Newall Research Fellow in Engineering, Inorganic Chemistry or Physics—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 30).

Agricultural Officer (Irrigation) (with a university degree in agriculture and preferably with experience in tropical irrigation research) in Kenya, to take charge of an irrigation research station and to be responsible for a research programme on irrigated crops and pastures—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/7/04/T.

Associate Professor or Assistant Professor (theoretical physicist, preferably with experience and main interest in the field of nuclear theory), to teach courses at the senior and postgraduate levels and to carry on research—The Head, Department of Mathematical Physics, University of Manitoba, Winnipeg, Manitoba, Canada.

Farm Manager (aged 25 to 35 years, with a university degree in agriculture) at Yundum Experimental Farm, Gambia, for technical and administrative duties in running an agricultural experimental station—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD/63/12/05/T.

Librarian—The Secretary, Oxford University Institute of Statistics, St. Cross Road, Oxford.

Master to teach Physics in the Upper School, including the Sixth Form—The Headmaster, St. Dunstan's College, London, S.E.6.

Organic Chemist (with a first- or good second-class honours degree and a good general knowledge of organic chemistry, and preferably postgraduate experience in steroid chemistry), to undertake the synthesis of labelled steroids—The Personnel Officer, The Radiochemical Centre, U.K.A.E.A., Amersham, Bucks, quoting Ref. No. 11/1959.

Research Assistants (2) (with a degree in chemistry or equivalent) in the Department of Chemical Technology, one for work on synthesis of nucleotides and analogous compounds and their biological application in cancer chemotherapy, and the other for work involving the synthesis of isotopically labelled organic compounds and the use of modern physical methods of analysis—The Registrar, Bradford Institute of Technology, Bradford 7.

Research Worker (with experience of research in the field of radioactivity), to be in charge of a newly established Isotope Unit—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

Senior Lecturer (with an honours degree or equivalent in mechanical engineering together with industrial experience) in the Department of Aircraft Propulsion—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

University Extension Lecturers (qualified archæologists specializing in the earlier prehistoric periods) in Archæology—The Deputy Director (Extension), University of London, Senate House, London, W.C.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Other Countries

Agricultural Research Council. A Bibliography of Farm Buildings Research, 1945–1958. Part 1: Buildings for Pigs. Pp. 36. (London: Agricultural Research Council, 1959.) 2*s.* 6*d.* [3011

Commonwealth Agricultural Bureaux. Plants Recorded as Resistant to Root-Knot Nematodes (*Meloidogyne* spp.). Catalogued by Dr. Mary T. Franklin and David J. Hooper. Pp. iii+33. (Farnham Royal: Commonwealth Agricultural Bureaux, 1959.) 7*s.* 6*d.* [3011

Forestry Commission. Forest Record No. 40: Preliminary Yield Table for Poplar. By J. M. Christie. Pp. 16. 1*s.* 6*d.* net. Forest Record No. 42: Use of Home-Grown Softwood in House Construction. By J. R. Aaron. Pp. 12. 1*s.* 3*d.* net. (London: H.M. Stationery Office, 1959.) [3011

Henry Wiggin and Co., Ltd. Publication No. 1122: Welding Nimonic Heat-Resisting Alloys for Gas-Turbine Assemblies. (Based on an article in *Aircraft Production*, July, 1959.) Pp. 19. (Birmingham: Henry Wiggin and Co., Ltd., 1959.) [3011

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 82: The Production of Clean Milk. Pp. 26. (Belfast: Ministry of Agriculture, 1959.) [3011

Department of Scientific and Industrial Research. Coalfield Papers of the Geological Survey of Great Britain No. 2: The Economic Geology of the Stirling and Clackmannan Coalfield, Scotland; Area South of the River Forth. By W. A. Read. With Palaeontology by R. B. Wilson. Pp. vi+73+2 tables and 15 figures. (Edinburgh and London: H.M. Stationery Office, 1959.) 7*s.* net. [3011

University of Leeds. Imperialism and Colonialism. By Prof. Raymond Aron. (Seventeenth Montague Burton Lecture on International Relations.) Pp. 18. (Leeds: The University, 1959.) 6*d.* [112

Henry Wiggin and Co., Ltd. Publication No. 1529A: Wiggin Nickel Alloys v. Caustic Alkalis. Pp. 51 (11 plates). (Birmingham: Henry Wiggin and Co., Ltd., 1959.) [312

Ten Years of Development in Cyprus: 1949–1959. Pp. 32. (London: Cyprus Government London Office, 1959.) [312

Other Countries

International Atomic Energy Agency. Nuclear Science Fellowships: Information—Opportunities—Procedures. Pp. 24. (Vienna: International Atomic Energy Agency, 1959.) [3011

Commonwealth of Australia: Department of Supply. Australian Defence Scientific Service: Defence Standards Laboratories. Report No. 242: The Laboratory Development and Evaluation of a Thixotropic Protective Oil for Critical Metal Surfaces. By E. C. Kuster. Pp. 1+17. (Maribyrnong, Victoria: Defence Standards Laboratories, 1959.) [3011

Report on the Geology of the Leeward and British Virgin Islands. By P. H. A. Martin-Kaye. Pp. iii+117+14 figures. (Castries, St. Lucia: Government Geologist, 1959.) [3011

Annual Report of the Calcutta School of Tropical Medicine and the Carmichael Hospital for Tropical Diseases, 1957–58. Pp. 146. (Calcutta: Calcutta School of Tropical Medicine and Carmichael Hospital for Tropical Diseases, 1959.) [3011

Fiskeridirektoratets Skrifter. Serie Havundersøkelser. Vol. 12, No. 4: A Note on the Effect of Electric Fields on *Gadus virens*. By Olav Dragesund and Helge Leivestad. Pp. 9. Vol. 12, No. 5: Scale Character and Growth Rate: a Study on Small Fat Herring from North Norway. By M. Soeprapto. Pp. 16. (Bergen: A. S. John Griegs Boktrykkeri, 1959.) [3011

Smithsonian Miscellaneous Collections. Vol. 139, No. 6: A Revision of the Silurian Bryozoan Genus *Trematopora*. By Richard S. Boardman. Pp. 14+2 plates. (Publication 4383.) (Washington, D.C.: Smithsonian Institution, 1959.) [3011

Dominion Museum Records in Ethnology. Vol. 1, No. 5: Maori Godsticks collected by the Rev. Richard Taylor. By Dr. T. Barrow. Pp. iii+183 (7 plates). (Wellington, New Zealand: Dominion Museum, 1959.) [3011

Chicago Natural History Museum. Fieldiana: Zoology. Vol. 39, No. 32 (September 30, 1959): Two New Game Birds from Peru. By Emmet R. Blake. Pp. 373–376. 10 cents. Fieldiana: Geology. Vol. 10, No. 34 (October 22, 1959): A Review of the Family Captorhinidæ. By Richard J. Seltin. Pp. 461–509. 1.25 dollars. (Chicago: Chicago Natural History Museum, 1959.) [3011

State of California: Department of Fish and Game. Fish Bulletin No. 107: Trout and Salmon Culture—(Hatchery Methods). By Earl Leftritz. Pp. 169. (Sacramento: Printing Division, Department of Fish and Game, 1959.) 2 dollars. [3011

United States Department of Commerce: National Bureau of Standards. Monograph No. 5: Preservation of Documents by Lamination. By William K. Wilson and B. W. Forshee. Pp. ii+20. (Washington, D.C.: Government Printing Office, 1959.) 20 cents. [3011

Machines and Tooling, Vol. 30, Issue No. 5. (Cover-to-cover translation of the Russian technical journal *Stanki i Instrument*.) Pp. 46+8. Published monthly. Annual subscription rates: Ordinary rate within the United Kingdom 70*s.*; Ordinary rate outside the United Kingdom 84*s.* Rate for Libraries of Educational Institutions within the United Kingdom 49*s.*; Rate for Libraries of Educational Institutions outside the United Kingdom 62*s.* (Melton Mowbray: Production Engineering Research Association of Great Britain, 1959.) [3011

Conseil Permanent International pour l'Exploration de la Mer, Charlottenlund Slot, Danemark. Rapport et Procès-verbaux des Réunions. Vol. 148: Contributions to Symposium on Salmon and Sea Trout, 1958. Pp. 80. (Copenhague: Andr. Fred. Høst & Fils, 1959.) Kr. 16.00. [3011

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